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STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE. I. The effect of alteration of hydrogen-ion concentration on the tone and contractions of plain muscle.

By C. LOVATT EVANS and S. W. F. UNDERHILL (*British Medical Association Research Scholar*).

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ALTHOUGH many investigators, *e.g.* Dixon, have recorded the effects of acid or alkali on plain muscle from various sources, yet in no published work which we have been able to trace has the effect of alteration of the hydrogen-ion concentration of the environment been studied from the general standpoint of the physiology of plain muscle. In most of the published work, the effects of change of reaction on the tonus, or on the spontaneous rhythmic contractions, of some particular preparation, have been studied. Even here, however, there is a lack of unanimity as regards the effects; the musculature of the blood vessels, for instance, is generally considered to be relaxed by increase, and constricted by decrease of hydrogen-ion concentration, and though there cannot be the slightest doubt that this is generally true, several investigators have obtained the opposite result. The explanation of this has been supplied by the careful researches of Fleisch, who has shown that in many of the perfused preparations employed for such experiments, the hydrogen-ion concentration and oxygen supply of the fluid used for perfusion were very abnormal, with the consequence that the vessels started in a state of maximal dilatation, which was not further influenced by small amounts of acid, but was replaced by constriction when an extremely acid reaction, far beyond any obtainable *in vivo*, was reached. Somewhat similar conclusions were reached by Atzler and Lehmann by the use of buffered perfusion fluids of known hydrogen-ion concentration. Whether the action is on the muscle tissue or on the nerves has not been finally decided.

We have therefore studied the question rather more fully, using various types of plain muscle, in order to ascertain how far, if at all, there was a general resemblance between plain and striated muscle in their behaviour towards change of reaction. The theoretical importance

of such an investigation is obvious; if the effects of acid on plain and striated muscle are similar, it might be reasonable to suppose that the mechanism of contraction is essentially the same in both types; but if the effects are fundamentally different then it must be inferred that acid production has, in plain muscle, no causal connection with contraction.

In carrying out these experiments, we have avoided very great changes in hydrogen-ion concentration, which can have little physiological significance. This probably explains why some of our results differ from those of previous investigators. There can be no doubt that contraction of many forms of plain muscle can be obtained by very great and sudden change of reaction, as by almost any other sudden change, chemical or physical. As instances of the effects of such sudden changes of reaction may be quoted some of the experiments on the uterus by Farndon and of one of us (C. L. E.) who found that the plain muscle of the heart of *Helix pomatia* went into tonus when treated with carbon dioxide.

According to Clark, living animal cells are not permeable to hydroxyl ions; if this quite probable view be accepted, then the effects which we have found must either be due to the hydrogen ion alone, or must be confined to alterations at the surface of the cells.

Methods. A few experiments on perfused blood vessels served to confirm the correctness of the conclusions drawn by Fleisch, and these need not be further referred to here. The tissues employed were the uterus and intestine of the cat, guinea-pig and rabbit, the sphincter of the iris of the cat, and the retractor penis of the dog. The method adopted was to suspend the portions of surviving plain muscle in a bath of some suitable saline solution at 38° C. in the manner described by Burn and Dale for the guinea-pig uterus. Except where otherwise stated, oxygen was bubbled at a constant rate through the saline solution in which the preparation was suspended. The record of the state of contraction of the tissue was made in most cases by the use of the frontal lever described by one of us (C. L. E.), but in some instances an ordinary tangentially writing lever was employed.

The reaction of the saline solution in the bath was determined at intervals by the use of the indicator method described by Dale and Evans, the procedure of dialysis being omitted. The reaction of the bath was altered, when required, by the addition of acid or alkali of such concentration (usually $n/10$) that the volume added was only 1–2 p.c. of the volume of saline already in the bath: it was found that unde these conditions it was immaterial whether the acid or alkali was mad

up with water or saline solution, or whether it was warmed or cold when added. The acids used were hydrochloric, phosphoric, sarcolactic, fermentation lactic, and carbonic. The last named was added either in the form of a saturated solution in saline solution similar to that in the bath, or by a carefully regulated stream of fine bubbles of the gas led into the bath at a slow and constant rate from a capillary delivery tube; regulation of the rate of flow was effected by a mercury column valve. As there was no essential difference between the effects of these different acids, we do not propose to describe the results obtained with each one; unless otherwise stated, it will be understood that the effects described apply equally to similar changes of hydrogen-ion concentration effected by any of these acids. Alteration in the direction of reduced hydrogen-ion concentration was brought about by addition of $n/10$ sodium hydroxide, or, in a few instances by sodium bicarbonate solution.

In some of the earlier experiments Ringer's solution of the composition given by Burn and Dale was used in the bath; it soon became apparent, however, that a stable reaction could not be maintained with this solution when oxygen was constantly passed through it, so a saline solution free from bicarbonate and lightly buffered with phosphate was subsequently used. This had the following composition: NaCl 8·5 grms., KCl ·42 grm., $CaCl_2$ ·24 grm., Na_2HPO_4 ·6 grm., molar H_3PO_4 ·2 to ·6 c.c. Distilled water, 1 litre (with or without addition of 1 grm. glucose). The distilled water was nearly always glass-distilled and free from carbon dioxide, but in a few of the experiments on the intestine ordinary distilled (condensed steam) water was used with the same results.

The reaction of the solution, as usually made up was about pH 7 to 7·3. This is much less alkaline than the usual Ringer bath after prolonged passage of oxygen bubbles. The phosphate solution cannot safely be made strongly alkaline, because of the ease with which calcium phosphate precipitates out from it, and this constitutes its chief drawback: solutions containing borate instead of phosphate were tried on this account, but were not found at all suitable.

The effect of mechanical conditions on plain muscle. It is well known that plain muscle is extremely sensitive to mechanical stimuli (Grützner); and Gôhara has found that the effect of mechanical stimulation plays an important part in experiments on the surviving vas deferens. In our opinion, the effects of mechanical stimulation are not sufficiently taken into account in using surviving preparations; some forms of plain muscle show contraction and others show relaxation as a result of mechaniacl stimuli. A most important factor is the mechanical effect produced by

the passage of the stream of oxygen bubbles through the bath in which the preparation is suspended. The guinea-pig uterus, which normally shows but little tone, responds by a prolonged contraction when the stream of oxygen bubbles is stopped. It might be supposed that this is due to oxygen lack, but such an explanation is not adequate, because if a stream of nitrogen or hydrogen be substituted for one of oxygen (which change itself causes some increase of tone), exactly the same thing occurs when the current of bubbles is interrupted (Fig. 1); the relaxed

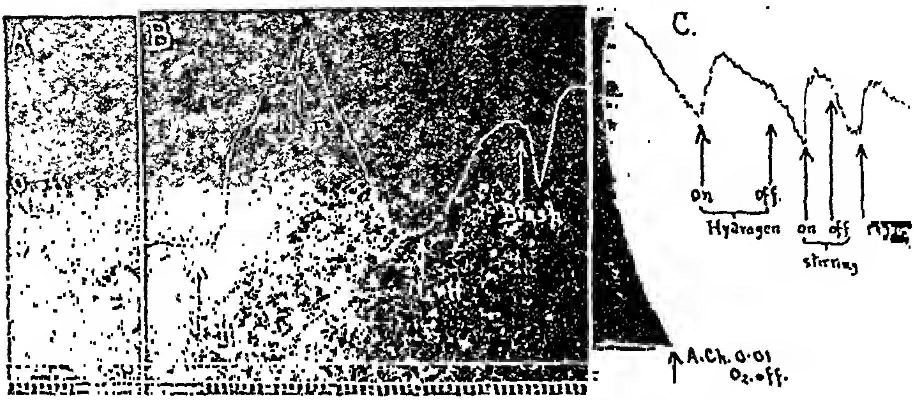

Fig. 1. Guinea-pig uterus—effect of agitation of the solution in the bath. *A.* Oxygen replaced by nitrogen bubbles. *B.* ·35 minutes after *A*, the nitrogen was turned off. *C.* Mechanical agitation on guinea-pig uterus contracted by acetyl choline.

condition is resumed when the bubbling of gas is recommenced, or if the liquid is agitated by stirring or if the surface of the tissue is gently stroked by a camel hair brush. Sometimes even briskly tapping the sides of the vessel with a glass rod is enough to produce the relaxation. It is curious that, when the tissue is in a contracted state, as in the presence of histamine or acetyl choline, the effect of the mechanical stimulation is reversed, since it now gives rise to the maximal state of tone, which becomes decidedly reduced when the stimulus due to the bubbling of oxygen, nitrogen or hydrogen is stopped (Fig. 1).

A similar phenomenon was shown by the guinea-pig intestine before and after the addition of acetyl choline. The cat's uterus, which is normally tonic, behaved, without addition of acetyl choline or histamine, in the same way as the tonically contracted guinea-pig uterus, *i.e.* mechanical stimuli brought about or maintained the contraction, but it was impossible to use nitrogen for this purpose, because oxygen lack speedily led to a relaxed state of the tissue, which then showed little

further change on stoppage of the current of gas; such change as there was, however, was in the direction of a further slight relaxation, and not contraction. We mention these phenomena to indicate that they play an important part in the experimental conditions, and further because we wish to anticipate false arguments causally connecting oxygen lack, and the lactic acid formation, which we believe to be consequent on it in this tissue, with the state of contraction.

Our opinion is, rather, that coutraction and tonus, and perhaps also relaxation, of plain muscle can be caused by the production of some metabolites. The effect of the stoppage of the stream of gas bubbles we imagine to be largely due to the consequent accumulation of such metabolites in the tissue, and it is significant that the guinea-pig uterus, when suspended in warm moist oxygen gas soon passes into a state of tonus, which we believe to have a similar origin. Not all forms of plain muscle behave in the same way, but a similar effect has been described by Mangold in stomach muscle, under the name of rigor mortis.

The small intestine. Our results in a long series of experiments on the ileum of the rabbit and cat have been generally concordant, though the magnitude of the effects obtained has been found to vary according to the physiological condition of the bowel, the extent and rapidity of the change in hydrogen-ion concentration, and the hydrogen-iou concentration existing at the moment the alteration was made. The cat's intestine was generally less sensitive to change of reaction than the rabbit's.

In the rabbit, starting from about pH 7·3, a sudden change to pH 6·5, or sometimes even to 6·8, stops the contractions and causes relaxation: the contractions may start again later, but the tonus is not usually restored. In the cat, a change to pH 6·5 causes slowing of the rhythmic contractions, and some loss of tonus, but a reaction of pH 6 or less is usually necessary to stop the rhythmic contractions (Fig. 2). In both cases, a change to the acid side of neutrality causes a decrease in the amplitude of the rhythmic contractions previous to their disappearance. A sudden change in the opposite direction, *e.g.* to pH 8, produces an increase of tonus, and again a reduction, though a less conspicuous one, in the amplitude of the rhythmic movements. Sometimes, especially when the tonus change is a steep one, there is a tendency for the effect to be only transitory, and partly to pass off afterwards. If the change in reaction is made quite slowly, *e.g.* over an interval of one or more minutes, the alteration of tone is either absent or is much less pronounced, and the effects on the rhythmic contractions are then more clearly seen. These appear at their best in a medium of which the reaction

is neutral, or very slightly on the acid side: a definite depression is seen at pH 6·5 and again at about pH 8; there is also commonly a slight

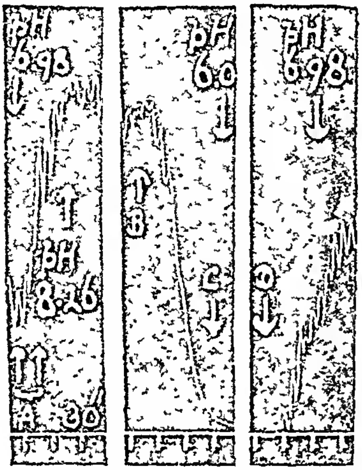

Fig. 2.

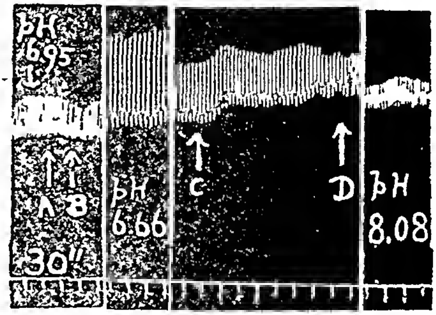

Fig. 3.

Fig. 2. Small intestine of cat. At *A*, 1·5 c.c. warm ·1 *n* NaOH. At *B*, carbon dioxide bubbled into bath; at *C*, CO_2 off; at *D*, contents of bath changed for fresh phosphate solution. Interval between portions of tracing, 3 minutes.

Fig. 3. Ileum of rabbit. Between *A* and *B* 4 c.c. of warm saline solution containing ·01 *n* HCl added. Between *C* and *D*, 7 c.c. dilute NaOH added gradually. Intervals between portions of tracing, from left to right = 17 mins., 8 mins., 9 mins.

alteration in rate in the direction of a slowing in an acid and a quickening in an alkaline medium (Fig. 3); in one experiment, for instance, a piece of rabbit's ileum showed 10 beats per minute at pH 6·58 and 13 at 7·74. So far as we could ascertain, the effects on the circular and longitudinal muscular coats were similar in all respects.

In one particular, we found a departure from the description given above, and this was with a rabbit's intestine in a bath the alkalinity of which exceeded pH 7·6, when a slightly relaxed state set in. When a sudden addition of acid was made, an increase of tonus appeared (Fig. 4). The same phenomenon was less clearly seen in some other instances when the pH exceeded 8. We regard this effect as due, not directly to the alteration of hydrogen-ion concentration, but to change in the ionic calcium content of the phosphate solution. At about pH 8 the liquid begins to show signs of turbidity, which rapidly increases as the alkalinity rises; this fine precipitate of calcium phosphate, which is especially liable to be produced when NaOH is suddenly added, leads to a reduction

in the calcium-ion concentration of the bath; indeed, it is not unlikely that, owing to the presence of small amounts of protein washed out from the tissue, which act as a protective, colloidal calcium phosphate is often present even before pH 8 is reached. We have not studied the effect of the calcium ion on plain muscle in any detail, but have observed that lack of calcium produces relaxation in the guinea-pig uterus; both Stiles and Fienga have observed that calcium in excess leads to a tonic contraction of the frog's and hen's œsophagus. The effect of acid in producing contraction when added to a bath of pH 8 or more does not seem to be at all surprising, since it would raise the calcium concentration.

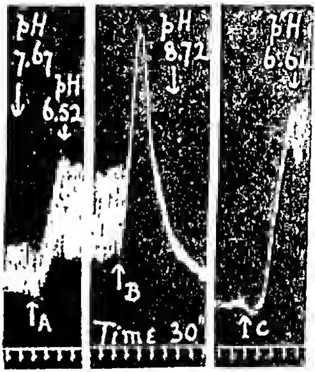

Fig. 4. Small Intestine of rabbit. At *A*, ·85 c.c. $n/10$ HCL At *B*, 1·5 c.c. $n/10$ NaOH. At *C*, 1·5 c.c. $n/10$ HCl. Interval between portions of tracing, from left to right = 8 mins., 7 mins.

The response of the intestine to pilocarpine was tested at various reactions: in neutral and alkaline fluids there seems to be little difference, but the response is definitely reduced in a bath of an acid reaction, and is abolished when the pH reaches 6. Extremes of reaction, *i.e.* beyond pH 8 and 6, rapidly kill the tissue, and it is interesting to note that when death is produced by the addition of acid, it happens with the tissue in the fully relaxed state.

Our results with the intestine agree generally with those of Hammett and of Bottazzi. Mansfeld and Hooker state that under certain conditions carbon dioxide can act as a stimulus to the intestinal muscle; in some of our experiments too, carbon dioxide has caused an increase of tonus, but we are inclined to attribute this exceptional result to change of calcium concentration, as explained above.

The guinea-pig uterus. The uterus of the non-pregnant guinea-pig proved to be much more sensitive to change of hydrogen-ion concentration than the small intestine, and owing to its greater instability, to give less concordant results. When the bath was definitely alkaline to begin with (pH 8) small additions of acid almost invariably produced a large contraction, which slowly declined and gave place to slow large rhythmic movements (Fig. 5). As the reaction of the original bath was

made less alkaline, this response became less pronounced, and when the initial reaction had reached pH 6 or 5, further acidification usually led

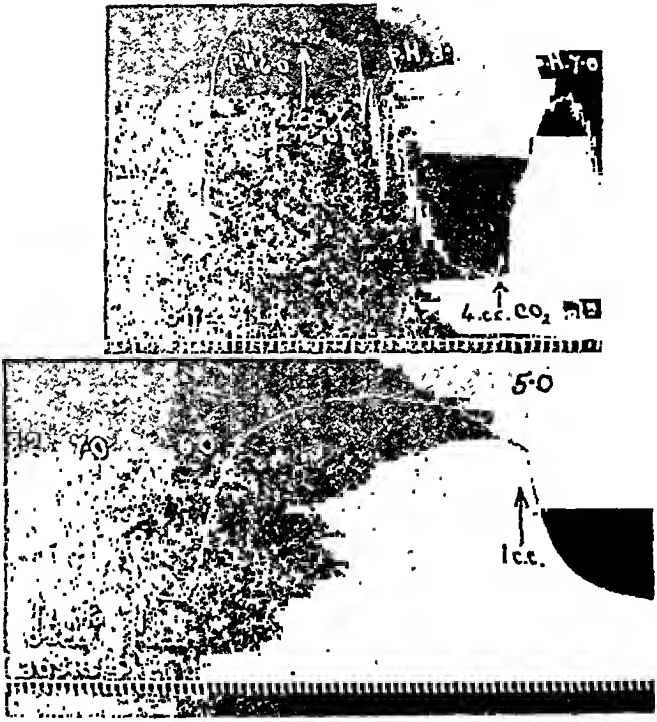

Fig. 5. Different effects of change of reaction on the guinea-pig uterus.

to a definite and rapid relaxation, with disappearance of the spontaneous rhythmic contractions (Fig. 5). In this relaxed state, the tissue is quite insensitive to even large doses of oxytocic drugs such as histamine; the tissue is not dead, however, as can be demonstrated by changing the contents of the bath back again to a solution of about neutral reaction, when the response to the drug returns in a short time (Fig. 6).

After a change of reaction (apart from excessive ones leading to temporary paralysis, such as that described above), the tissue slowly settles down again and becomes more or less quiescent under the changed conditions at an approximately normal extended length. When this has happened (which often takes an hour or so), it is found that the response to drugs is unchanged. Fig. 7 illustrates the constancy of response to ·01 mg. of histamine at pH values from 6·5 to 9·2 (bicarbonate Ringer). The effect of alkalies is the reverse of that of acids (Fig. 5).

The general effect of alteration of reaction is similar to that seen in the experiments on the intestine, but the contraction seen on addition of acid to a preparation in an alkaline bath is much more pronounced with the uterus. We believe the explanation to be similar, and the effects to be more conspicuous because this tissue is more

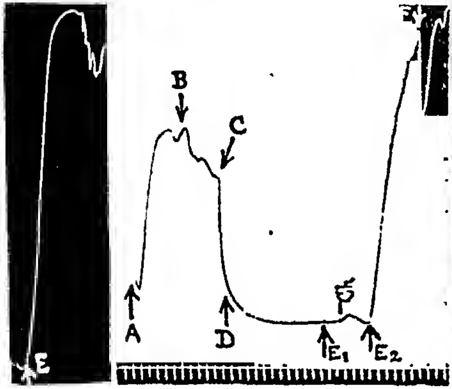

Fig. 6.

Fig. 7.

Fig. 6. Guinea-pig uterus. First portion shows the normal response to ·1 mg. histamine. At *A*, ·25 c.c. 5 p.c. lactic acid; between *B* and *C*, 15 c.c. saturated CO_2 solution, and at *D*, ·5 c.c. 5 p.c. lactic acid. The reaction was now about *p*H 5. At E_1, ·1 mg. histamine produced no effect. The contents of the bath were then changed, and at E_2, ·1 mg. of histamine produced the usual effect. At *F*, ·5 c.c. saturated CO_2 added produced a transitory intermission of the contraction.

Fig. 7. Response of guinea-pig uterus to ·01 mg. histamine at different reactions.

sensitive to an alteration in the ionic calcium content of the fluid in which it is immersed.

The uterus of the rabbit and cat. Our results on these tissues agree with those of Farndon on the cat's uterus. The plain muscle of these organs and particularly that of the cat, differs from that of the guinea-pig uterus in exhibiting a more definite tonus when placed in baths at ordinary reactions. The response to acidification was in every case a relaxation of tonus, and usually at first a slowing and amplification of

the rhythmic contractions: with further increase of hydrogen-ion concentration the rhythmic movements slowly faded away (Fig. 8).

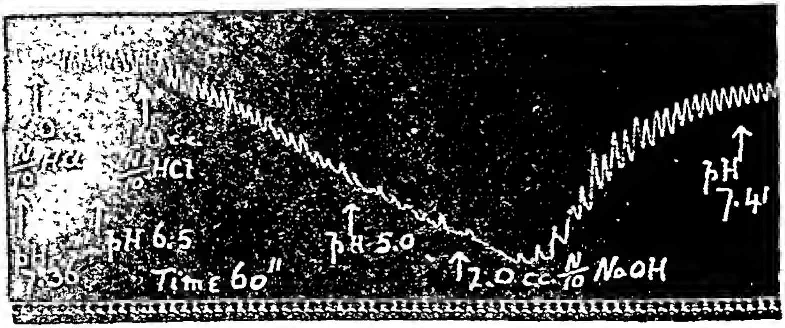

Fig. 8. Effect of change of reaction on the cat's uterus.

The retractor penis and iris. Mosso has stated that pure carbon dioxide causes contraction of the retractor penis of the bullock, while Bottazzi states that carbon dioxide diminishes the tonus of the dog's retractor. Our results are at variance with those of both observers, since we have never observed any effect on moderate change of reaction. We should not have expected to see any relaxation, because the retractor penis, in our experience, does not normally display tonus. The same remarks apply to the isolated iris of the cat, which we tried on two occasions, with negative results.

The influence of local nervous mechanisms. The old and obvious objection that different responses in plain muscle may be attributed to varying effects on the nervous elements present in the preparation leaves us in somewhat of a quandary in interpreting our results; we have tried by various methods to circumvent these objections, in order to be able to relate the results to effects upon the muscle alone. It is difficult, however, or even impossible, entirely to dissociate the two, as a consideration of the state of affairs in the cat's uterus will show. According to Langley and Anderson the nerve supply to this organ is a sympathetic one, and their experiments also give evidence for the existence of some terminal cell stations in the organ itself. Dale and Cushny found that the principal effect of hypogastric stimulation on the virgin uterus was inhibitory, but that in the pregnant uterus the principal effect was motor, due, according to Cushny, to the predominating effect of motor fibres in the normal nerve supply. Both the motor and the inhibitory effects of hypogastric stimulation were abolished by nicotine in large doses, but were unaffected by atropine. If we assume that

the normal tonus of the cat's uterus is of nervous origin, it would seem not unreasonable to suppose that the action of increased hydrogen-ion concentration is akin to that of adrenaline, and stimulates the inhibitory nervous structures; in any case, acid would presumably show no effect at all in the absence of the initial tonus, which it is able to relax. So that the nervous mechanism in the tissue would on this assumption, be at least indirectly responsible for the effect produced by the addition of acid, or even directly responsible for it, if we suppose it to act by exciting the inhibitory nerves.

We have studied the effect of alteration of reaction upon cats' uteri previously treated with nicotine or atropine; we used the latter because of the statement made by Schultz and other authors that atropine paralyses all the nerve endings in plain muscle (Grützner, p. 59). It was found that the effect of change of reaction in presence of ·1 p.c. nicotine or ·01 p.c. atropine was essentially the same, as regards the effect on the tonus, as in the absence of these substances, which would lead us to infer that the relaxation is a real effect on the muscular tissue. Further, we have observed the responses of cats' uteri preserved at 0° C., from day to day, until the tissue was dead. The response was never altered in direction, but merely in magnitude, as the vitality and normal tonus of the tissue slowly fell off as a result of keeping, until finally the tissue ceased to react to all stimuli. It would seem very unlikely that nerve cells and plain muscle tissue should exhibit exactly the same degree of resistance to cold, and perish simultaneously, so that we are probably justified in regarding the response throughout as due to effects on the muscle itself.

Similar experiments were carried out on the isolated small intestine: the response to change of reaction is unaltered by nicotine, or by preservation at 0° C., which in one case extended up to 8 days. Although Cannon and Burkett have shown that the myenteric nerve plexus is much more resistant to anæmia and low temperatures than might have been imagined by analogy with other nerve cells, the same argument applies here as to the cat's uterus. Hammett has found that the contraction of the rat's intestine produced by sodium carbonate is abolished by cocaine, but not by nicotine; he concludes that the action is on the nerves.

An attempt was also made to decide the question by reference to strips of the inner part of the circular muscle coat of the intestine prepared by the method of Gunn and Underhill. Although nerve cells are not considered to penetrate the circular muscle coat, the strips of

muscle after use were fixed, serially sectioned, stained with Pappenheim's stain, and examined for the presence of nerve cells. In some preparations one or two nerve cells were seen, but, so far as our examinations went, these were not regularly present. The isolated rings or strips of intestinal muscle are very sensitive to change of reaction, and rarely recovered from any considerable alteration, so that the results are somewhat ambiguous: also, we have never seen any alterations of tone at all in these preparations, but only in the rate and amplitude of the rhythmic contractions. The handling of the tissue involved in the removal of the rings of muscle is rather severe, and it may be that the natural tonus of the tissue has suffered in consequence, so that the effect of acid in causing relaxation was not seen as with the intact intestine, which had been less roughly treated. When there is no natural tonus in a preparation it is clear that a relaxation effect cannot be demonstrated: for this reason, the retractor penis showed no effect with the moderate changes of reaction which we have used. Incidentally, this form of plain muscle is, according to Fletcher, devoid of nerve cells. The same is true of the cat's iris, in which also acids produced no effect.

Comparison of effects of change of reaction on plain with those on striated muscle. As compared with plain muscle, striated muscle appears

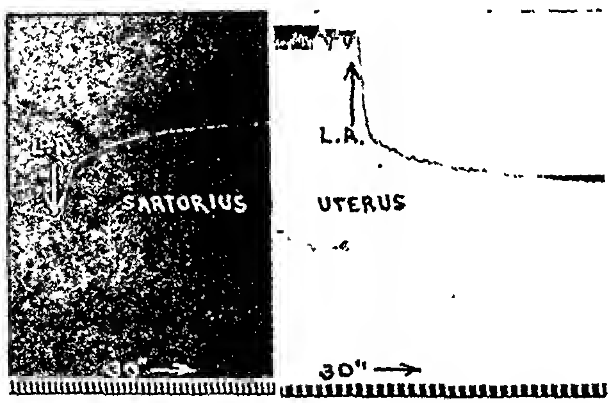

Fig. 9. Death of striated and plain muscle by acidification compared. In each case the bath was made up to a lactic acid content of ·27 p.c.

to be much more indifferent to small changes of hydrogen-ion concentration: when the acidity passes a certain limit, however, rigor rapidly sets in, and the muscle shortens. Plain muscle behaves in an entirely different way: any tonus it may have is, with certain exceptions, explicable in other ways, reduced by addition of acid, so that ultimately

in all cases, a fully relaxed state is reached, in which all spontaneous movement is abolished, and in which the tissue is unresponsive to drugs normally producing contraction; further addition of acid soon leads to death, but this occurs in the relaxed state, and is not followed by any shortening. This result is clearly shown in Fig. 9, where the effect of equal acidification of striated and unstriated muscle from the same animal is shown. In both cases 3 c.c. of normal sarcolactic acid was added to the bath of 100 c.c. of Ringer's solution containing the tissue, so that the ultimate concentration of lactic acid in the bath was ·27 p.c. Death of the plain muscle was confirmed by changing the fluid for the usual saline solution, and showing that addition of ·25 mg. of acetyl choline was without effect.

Conclusions.

1. The effect of increase of hydrogen-ion concentration, within limits compatible with life, is to cause relaxation of the tone of plain muscle. Rhythmic contractions, such as those of the small intestine, are slowed and depressed in extent on the acid side of neutrality, and quickened and depressed when the reaction becomes decidedly alkaline. Excessive acidification leads to death in a relaxed state.

2. In muscle preparations devoid of tonus, increase of hydrogen-ion concentration has no effect.

3. These effects are independent of action on local nervous mechanisms.

4. When the preparation is immersed in a definitely alkaline bath of phosphate solution (pH 8 or more), addition of acid often produces an increase of tonus, or a large contraction. This effect, well seen with the guinea-pig uterus, is believed to be due to alteration in the ionic calcium content of the fluid, and not directly to change in the hydrogen-ion concentration.

5. The mechanism of contraction and tonus in plain muscle is different from that in striated muscle.

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ISOLATION OF DIFFERENT PARTS OF THE DIGESTIVE TRACT AS A METHOD OF STUDYING ITS MOVEMENTS. By B. P. BABKIN AND E. I. SINELNIKOV.

(*From the Physiological Laboratory, University of Odessa.*)

For the purpose of investigation of intestinal movements we have tried several forms of establishing a permanent fistula and have finally adopted the method here described.

Two consecutive operations were performed on a dog. The first consists of a lateral anastomosis of the small intestine in the upper part. The resulting semi-isolated intestinal loop should be about 20–25 cm. long and must be well supplied with blood vessels. The second operation is performed after an interval of three weeks, when the completion of the separation of the intestinal loop formed in the first operation is effected. The intestine is crushed by means of an enterotribe near the anastomosis and then cut transversely. This leaves four loose ends of the intestine: two ends adjacent to the anastomosis and two others belonging to the separated loop. All four ends are now closed by sutures. The continuity of the digestive tract is thus preserved and at the same time a blind intestinal loop hanging on its mesentery is formed. A metal cannula (10–11 mm. bore) is now introduced into one of the two ends of the separated loop. The loose end of the cannula is brought to the skin surface by means of a trocar and fixed to the abdominal wall in the usual manner. If the cannula is introduced in the distal end of the isolated loop it is advisable to fix with a few stitches its proximal end to the inside surface of tho abdominal wall. This will effectively prevent any possible intussusception (Fig. 1).

An animal thus operated upon is ready for the experiments about 10 days after the second experiment. It should be borne in mind, however, that the secretory and the motor activities of the isolated portion of the intestine are greatly increased during the first two to three weeks.

The following two methods were used by us for registration of the movements of the intestinal segment.

(1) Air transmission. A Marey's tambour or a piston recorder is connected by means of rubber tubing, directly to the fistula tubing. The

secretion of the intestinal juice can be measured simultaneously with the registration of the movements. The cork which closes the fistula

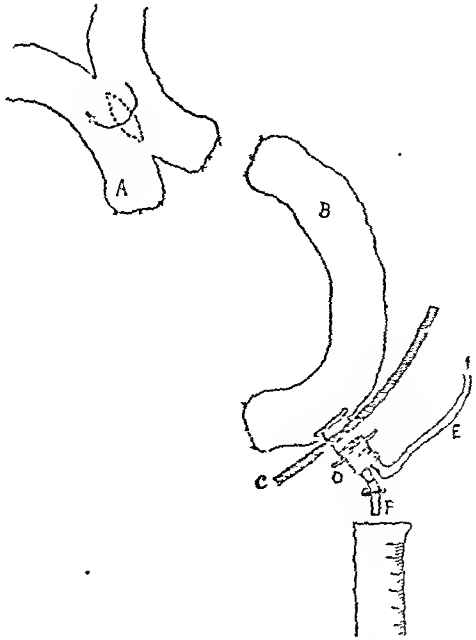

Fig. 1. *A.* Enterostomosis as result of first operation.
B. Isolated loop of intestine.
C. Abdominal wall.
D. Cannula.
E. Tubing leading to the registering apparatus.
F. Tubing through which the intestinal juice drops into a cylinder.

tubing is provided for this purpose with two glass tubings, one connected to the registering apparatus, the other leading to a graduated cylinder to collect the juice (Fig. 1).

(2) The filling method. The intestinal segment is filled with Ringer's fluid or other fluid the action of which is to be tested. The filling is performed under a definite pressure, since a greater pressure than 20–40 mm. of water sets up rhythmic movements.

Changes of the intra-abdominal pressure due to coughing, vomiting, and to a lesser degree to the movements of the animal, are also recorded in both cases of transmission, but all these movements can be easily recognised by their transitory character and can never be mistaken for true intestinal contractions. The respiratory movements can be controlled by their simultaneous registration.

The method has the following advantages over those previously used: (*a*) The animal is kept in a perfectly healthy condition for an appreciable length of time. (*b*) The intestinal loop being in connection with the remaining organism at the same time is easily available for manipulations. (*c*) The method allows us to distinguish between the mechanical and the chemical effects of the substances introduced into the loop. (*d*) Although the tracings obtained by means of this method represent a total effect of several factors, nevertheless peculiar kinds of movements, such as those of peristalsis and segmentation, give quite typical forms of curve. (*e*) Not only the movements but also the secretion of the isolated intestinal loop can be studied.

A post-mortem examination of a dog, which accidentally died eight months after the operation, was performed. The mucous membrane and possibly the muscular layers of the isolated intestinal segment seemed to be in a condition of slight atrophy. No weakening of the motor and secretory activity of the loop was however observed during the life of the animal. This fact coincides with Cash's(1) previous observation. It is possible that by stimuli repeated sufficiently often both glands and muscle can be kept in a completely normal condition, for Savich(2, 3) has shown that in Thiry-Vella fistula glands which have been at rest for years began to secrete soon after the application of mechanical stimuli and secreted still more freely after the introduction of pancreatic juice.

In addition to the isolation of segments from the small intestine, attempts were made in our laboratory to isolate by the same method sections of the large intestine. The results obtained by this method will form the subject of a further communication.

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ON THE ABSORPTION OF INSULIN FROM THE STOMACH. By L. B. WINTER.

(From the Biochemical Laboratory, Cambridge.)

DUDLEY(1) has recently shown that insulin is rapidly destroyed by pepsin and trypsin *in vitro*. It is probably for this reason, rather than inability of insulin to pass the intestinal wall, that administration of pancreatic extracts by the mouth has been relatively ineffective. Sutter and Murlin(2) showed that pancreas extract given by the mouth to a diabetic patient was without effect, while subcutaneous injection of the same extract was successful. Murlin, Clough, Gibbs, and Stokes(3) have shown that the respiratory quotient in depancreatised dogs could be raised if extracts of pancreas dissolved in $N/20$ NaOH together with glucose, were given by the stomach tube. Since the acid of the gastric juice is not sufficiently strong to destroy insulin, the purpose of the alkali was "not for the purpose of neutralising the acid *per se*, but for the purpose of inactivating pepsin." At least $12\frac{1}{2}$ units were necessary to cause an increase in the R.Q. which could be detected. Insulin is considerably adsorbed in acid solution, but only to a small extent when the reaction is alkaline (Dudley). This may be a reason for the absorption of insulin from the stomach when given in alkaline solution as in Murlin's experiments. It is known that water is not, and that alcohol is absorbed from the stomach. It seemed possible, therefore, that administration of insulin by the mouth might be more successful if it were given in weak alcoholic solution.

Rabbits were used throughout. It is well known that these animals are very susceptible to nervous influences, and since the blood sugar was to be studied care was taken to accustom the animals to the stomach tube. It seemed also desirable to determine whether alcohol by itself might cause any appreciable alteration in the amount of the sugar in the blood. 4 or 5 c.c. of 95 p.c. alcohol were diluted to 20 or 25 c.c. with water, warmed to body temperature, and introduced into the stomach through a rubber tube. Though the blood sugar was only slightly affected, in a variable direction, the animals soon became unable to stand, and narcosis supervened. For this reason the animals used in the main experiments were accustomed to the absorption of alcohol by means of

gradually increasing doses on successive days, until 4 or 5 c.c. of 95 p.c. alcohol (suitably diluted) could be absorbed without interfering symptoms occurring as a result of the alcohol.

Bang's old method was used for the blood sugar determinations. Blood was usually obtained from the marginal vein of the ear. In one case only was it drawn from the heart. The animals were kept from food for twenty-four hours previous to the administration of insulin. The activity of the crude insulin used was such that the subcutaneous injection of 40 mg. into a rabbit weighing 1·5 kg. caused convulsions in two and a half hours. The insulin was dissolved in 15 to 25 c.c. of 20 p.c. alcohol, warmed to body temperature, and administered by the stomach tube.

In the first experiments large amounts of insulin or yeast extract were used, with the result that the blood sugar quickly fell to the convulsion point (·04–·05 p.c. by Bang's method) It was evident, therefore, that rapid absorption of the insulin must have taken place from the stomach. In those cases in which convulsions occurred, no difficulty was experienced in recovering the animals with glucose. Experiments were then carried out using smaller quantities of insulin. 60 mg. had an appreciable effect, and in one experiment half this amount reduced the blood sugar to ·04 p.c. though convulsions did not actually occur, and the animal recovered without the aid of glucose. The variable response of different animals to subcutaneous injection of insulin is well known and causes considerable uncertainty in the testing of preparations. A still greater uncertainty must attach to those experiments in which the extract is administered per os. The blood sugar values show, however, that the effect of insulin on animals is similar, whichever of these two methods is used. When large doses were given the blood sugar was reduced very rapidly, with smaller doses the fall and rise were both slower. A delayed action following the administration of insulin per os has not been noticed. In no case has the action been prolonged as often occurs when a subcutaneous injection of yeast extract has been given. Presumably the insulin is destroyed in the intestine after a certain lapse of time, when most of the alcohol has been absorbed. That the alcohol was responsible for the absorption of the insulin was shown by a control experiment in which 60 mg. of insulin were given per os dissolved in normal saline. No effect on the blood sugar was observed. Another experiment in which the same amount was given per os dissolved in $N/20$ NaOH gave negative results.

In view of the considerable quantity of undigested food necessarily

present in the animals' stomachs, it seemed unlikely that any advantage would result if insulin hydrochloride (Dudley) were used instead of crude insulin. The interfering substances in crude insulin are of small account in such a method of administration. The experiments performed with the hydrochloride tend to support this view. 2 mg. of the sample of hydrochloride used caused convulsions in two hours when injected subcutaneously into a 1·5 kg. rabbit. 3 mg. caused only a moderate fall in the blood sugar when administered in alcohol per os.

Summary.

1. Insulin in weak alcoholic solution administered to rabbits per os causes the blood sugar to fall to a low level.

2. This effect was not observed when similar amounts of insulin were dissolved in normal saline, or in $N/20$ NaOH.

I wish to thank the Medical Research Council for a personal grant held during the course of this work.

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PROTOCOLS.

(The following are typical experiments.)

Rabbit 1·6 kg.

Time	9.45	Blood sugar	·09 p.c.
	10·00	90 mg. insulin per os in 20 c.c. 20 p.c. alcohol	
	11.00		·04
	12.30		·04
	2.30		·07
	4.15		·10

Rabbit 1·5 kg.

Time	10.00	Blood sugar	·09 p.c.
	10.20	60 mg. insulin per os in 20 c.c. 20 p.c. alcohol	
	11.30		·05
	12·15	Animal collapsed	
	12.30		·03
	12.40	40 c.c. 10 p.c. glucose injected subcutaneously	
	1.00	Animal eating	
	2.40		·10

Rabbit 1·6 kg.

Time 9.45		·12 p.c.
10.00	60 mg. insulin per os in 20 c.c. 20 p.c. alcohol	
11.20		·09
1.00		·08
3.00		·10
4.30		·11

Rabbit 1·4 kg.

Time 9.45		·12 p.c.
10.00	3 mg. insulin HCl per os in 20 c.c. 20 p.c. alcohol	
11.20		·08
1.00		·09
2.30		·10

Rabbit 2 kg.

Time 9.45		·11 p.c.
10.00	60 mg. insulin per os in *N*/20 NaOH	
11.30		·11
1.00		·10
2.30		·11
4.00		·11

Rabbit 1·4 kg.

Time 10.00		·10 p.c.
10.15	30 mg. insulin per os in 15 c.c. 20 p.c. alcohol	
11.00		·06
1.00		·05
2.30		·04
4.30		·07
6·00		·09

Rabbit 2 kg.

Time 10.00		·10 p.c.
10.10	60 mg. insulin per os in 20 c.c. normal saline	
11.15		·10
12.30		·11
2.20		·10

Rabbit 1·7 kg.

Time 10.30		·08 p.c.
10.45	20 c.c. 20 p.c. alcohol per os	
11·30		·07
1.00		·08
2.45		·08

STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE.
II. The Oxygen Usage of Plain Muscle, and its Relation to Tonus. By C. LOVATT EVANS.

(*From the National Institute for Medical Research, London, and the Physiological Laboratory of St Bartholomew's Hospital.*)

THE gaseous metabolism of organs rich in plain muscle has been the subject of several investigations. Brodie and Vogt(1) determined the exchanges of the small intestine by Barcroft's method, and found the oxygen usage to be about ·018 c.c. per gram per min. (= 1·08 c.c. grm./hr.) when the intestine was at rest; deducting ·026 c.c. per gram per min. for the mucosa, leaves about ·01 c.c. per gram per min. (= 0·6 c.c. grm./hr.), if we assume that half of the weight of the organ is made up by muscle. The R.Q. was about 1·1. Cohnheim and Pletnew(2) found that the intestine produced about 85 mg. CO_2 per 100 grams per hour, which I calculate to be about ·007 c.c. per gram per min. (= ·48 c.c. grm./hr.) Parnas(3), by indirect methods, estimated that the oxygen usage of the adductor muscle of a bivalve was not much greater when the tonic muscle supported a weight of 3 kg. than when it was in the relaxed and unweighted condition: these results were supported by Bethe(4) on theoretical grounds, and further by experiments on *Unio* and *Aplysia*. The experiments, however, were rather indirect, and their results might reasonably be questioned, as has been done by Cohnheim(5), Cohnheim and v. Uexkull(6) and Noyons and v. Uexkull(7). Since similar results have been claimed for striated muscle *in vivo* in a tonic state (Roaf(8)), the general principle of the metabolism of plain muscle in the relaxed and tonic states seemed well worth investigation.

Methods. Isolated surviving tissues were used in all the experiments. The tissues were the uterus and the small intestine of various animals. The mucosa was in some cases left intact, in others removed. It was found better in the experiments in which it was desired to compare the tonic with the relaxed state, to leave the mucosa intact, and to damage the tissue as little as possible. The tonic state was induced by the action of drugs, such as pilocarpine, histamine or acetylcholine, and the relaxed state by the action of relaxing drugs when this was necessary.

Two different methods were employed for the determination of the

oxygen usage; both gave similar results, but it was found advantageous to have the two methods available, because each of them presented certain drawbacks which were obviated by the other.

The first method consisted in a simple modification of the micro-respirometer described by Winterstein, and subsequently improved by Krogh(9). In order to permit of the taking of a record of the state of tonus of the muscle during the experiment, the arrangement shown in Fig. 1 was employed. The micro-respirometer was filled with warm moist oxygen in the usual manner, the whole apparatus, with the exception of the manometer, being immersed in a well-stirred thermostat, the temperature of which was kept constant to within ·02° C. by a toluene regulator. The plain muscle *M*, was fastened to a platinum needle fixed into the connection tube of the container; to the other end of the muscle a human hair was attached; this passed through a tube about 2 mm. internal diameter and over two light pulleys to a recording lever. To render the tube which gave passage to the hair gas-tight, its lower end was made extremely narrow, and contained a drop of mercury, which was retained by surface tension: above the mercury the tube was filled with vaseline, which at the temperature of the bath was fluid, but sufficiently viscous. A small amount of a drug in solution in *H* could be run over the surface of the muscle by connecting *F* with an oxygen gasometer, opening tap *A*, connecting taps *B*, *C* and *D*, so as to have a direct way through from *F* to *E*, and then carefully turning on the oxygen current until about 1 c.c. of water rose into the bulb of *E*. The taps *A* and *D* are then closed, *G* momentarily opened to equalise pressures, and *C* and *B* turned to put the two vessels into connection with the manometer. The small volume of fluid expelled from the tube *H* through *A* passes by a wick, shown in the figure as a dotted line, and flows over the surface of the tissue. This apparatus was constructed and used in 1919, and almost simultaneously a somewhat similar, and I think in some ways better, modification of the micro-respirometer was described by Adam(10). This apparatus had the disadvantage that there was no arrangement for washing away metabolites from the tissue: these accumulated and themselves produced alterations of tonus (*e.g.* a contraction in the case of the guinea-pig uterus), which obscured the results: the tonus could, however, in this tissue, be relieved for a time by running adrenaline over the preparation.

The second form of apparatus used overcame these defects, but introduced others, particularly that of diminished accuracy. It consisted of a modified Ringer-bath in which the tissue was immersed; the

oxygen-content of the Ringer's solution was determined at the commencement and at the end of a period of observation. The apparatus is

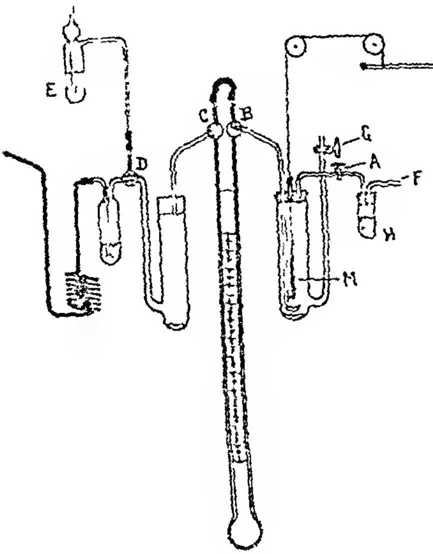

Fig. 1.

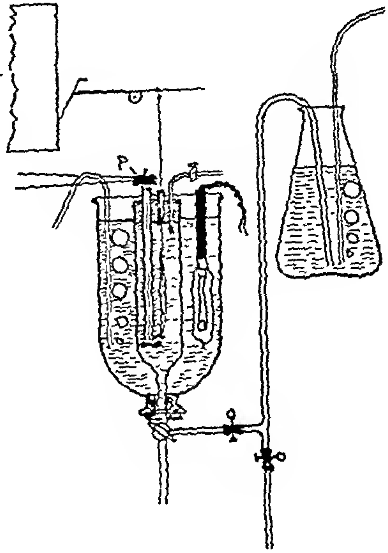

Fig. 2.

shown in Fig. 2. An outer glass vessel was heated by an electric lamp immersed to a suitable depth in the water, which was stirred by a stream of air bubbles. The inner vessel, containing the plain muscle, was rapidly stirred by the small silver paddle connected to the pulley *P*. (This stirring is absolutely essential for reasons given by Evans and Underhill(11).) The shaft connecting the pulley with the paddle was of silver, and almost filled the bearing tube, so as to exclude contact with the air as much as possible. The plain muscle was fixed to a platinum pin fixed on the bearing tube, and its upper end was hooked to a small silver pin attached to a human hair; the hair passed through a fine-bore tube to a frontal writing lever, by which the state of contraction of the tissue was recorded on a revolving smoked drum. A third tube leading through the cork closing the inner vessel served for the introduction of small volumes of drug solutions.

Warmed oxygenated Ringer's solution was siphoned over from a storage vessel kept at 40° C., and through which a constant stream of oxygen bubbles was passed, into the container at the commencement of each period of observation, until it overflowed from the middle tube of the apparatus. After allowing a few moments for admixture, a sample of about 25 c.c. of the fluid was withdrawn for the oxygen estimation, and

the container at once refilled by the siphon: alternatively, the container was filled and the sample for analysis taken from the siphon tube immediately afterwards. This latter was theoretically the better procedure, as the inner container had a capacity of only 39 c.c., so that the withdrawal of the sample almost emptied it, but in practice equally good results were obtained by either method. The oxygen estimations were made by a micro-modification of Winkler's method (12). Periods of observation were usually of 20 or 30 minutes' duration, at the end of which time a second sample was taken for analysis. In an earlier form of the apparatus two containers were used, one for the plain muscle and one for a control, but the latter was subsequently found to be unnecessary.

The oxygen usage of surviving plain muscle. When the oxygen supply to the tissue is adequate, and the conditions uniform, the rate at which oxygen is consumed by surviving plain muscle preparations is reasonably constant whichever method is used: this is illustrated by Exps. 1 and 2.

Exp. 1. Guinea-pig uterus. ·37 gram. Micro-respirometer method.

Period	Duration, mins.	c.c oxygen per grm. per hr.	
1	20	·39	Maximum deviation from mean = 5 5%
2	10	·36	
3	10	·37	

Exp. 2. Rabbit uterus. 1·89 grams. Bath method, immersed in Ringer's solution.

Period	Duration, mins.	c.c oxygen per grm. per hr.	
1	20	·30	Maximum deviation from mean = 10 5%
2	20	·29	
3	20	·26	
4	30	·31	

It is, I think, right to state that the results obtained by the use of the micro-respirometer are usually correct to within less than 10 p.c., and those obtained by the bath method to within 15 p.c. This degree of accuracy is enough for the purpose of the present series of experiments, in which greater variations than 20 p.c. are sought for. The adequacy of the oxygen supply is an essential condition for satisfactory results; this is more easily attained when the micro-respirometer is used than with the bath method, because in the latter oxygen is being continually used up, and the oxygen pressure is steadily falling from the commencement of the period of observation. This fall of oxygen-pressure is further aggravated by local oxygen deficit if the stirring of the bath is not constantly and efficiently performed. When oxygen deficit occurs, it results in reduced oxygen intake by the tissue, and this becomes more apparent the longer the period of observation. It is therefore a good safeguard, when using the bath method, to avoid letting the final oxygen

content of the bath fall too low, and, if oxygen deficit is suspected, to run one period for a longer time than the others, in order to see whether it shows a diminished oxygen usage in unit time. In period 4 of Exp. 2, for instance, there was no falling off in the oxygen rate although the period was half as long again as the preceding ones; the oxygen supply was therefore adequate for a period of this duration, although a calculation shows that the oxygen-pressure during the period fell from 610 mm. Hg to 355 mm. Another factor which greatly affects the accessibility of the tissue for oxygen, is the size of the piece of tissue, as has been shown by Krogh for skeletal muscle(13): for this reason large masses of tissue were always avoided, and long thin organs, such as the guinea-pig uterus, or strips of intestine were found to give the best results.

The results obtained are best exhibited in a table, and Table I gives such a summary of experiments with tissue from various sources, both in the tonic and relaxed conditions, while Exp. 3 gives further details of an experiment in which the state of tonus was altered by the addition of the drugs (bath method), and Fig. 3 shows parts of the record obtained in this experiment.

Exp. 3. Rabbit uterus. 1·89 grams.

Period	Duration, mins.	Contents of bath	State of tone	c.c. O_2 per grm. per hr.
1	20	Ringer	Normal	·30
2	20	,,	,,	·29
3	20	,, + ·2 c.c. 1 : 5000 adrenaline	Increased	·25
4	20	,,	Normal	·26
5	30	,,	,,	·31
6	20	,, + ·1 mg. histamine	Increased	·23
7	20	,, + n/10,000 NaCN	Diminished	·22

TABLE I. c.c. oxygen per gram per minute by tissue.

Exp.	Tissue	Tonus reduced	Tonus normal	Tonus increased	Method
4	Guinea-pig uterus	—	·46	·49 (histamine)	Micro-respirometer
5	,, ,,	—	·54	·57 (,,)	,,
6	,, ,,	,,	·15	·13 (,,)	Bath
7	Rabbit vagina	—	·26	·25 (adrenaline)	,,
8	,, intestine	·79 (atropine)	·88	·68 (pilocarpine)	,,
9	Guinea-pig intestine	—	1·43	1·11 (acetylcholine)	Micro-respirometer
10	Cat ,, (muscle only)	·18 (adrenaline)	·15	·13 (pilocarpine)	Bath
11	Cat uterus	·79 (,,)	·35	·12 (histamine)	,,
12	Rabbit uterus	—	·29	·25 (adrenaline)	,,
13	Guinea-pig uterus	—	·43	·38 (histamine)	Micro-respirometer
14	,, ,,	·29 (adrenaline)	·36	·30 (acetylcholine)	,,
15	Rabbit uterus	—	·29	·23 (histamine)	Bath
		Mean	·46	·39	

The results show that in the tonic state not more, but less oxygen is consumed, than by the same tissue when in the relaxed condition.

It might be supposed where relaxation was produced by addition of adrenaline, as with the small intestine, or cat's uterus, that the result

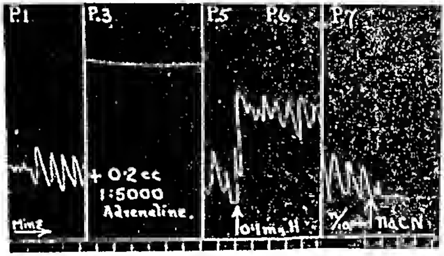

Fig. 3.

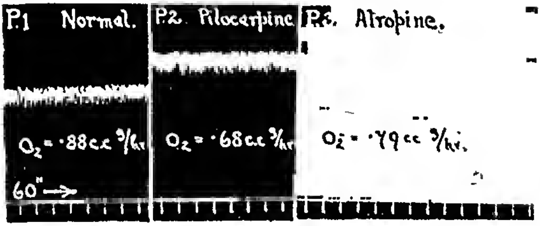

Fig. 4.

might be influenced by the rapid absorption of oxygen by the adrenaline, which would increase the apparent oxygen usage of the tissue. This objection is, however, unfounded, as a simple calculation shows: if we assume that in the course of its spontaneous oxidation a molecule of adrenaline uses 3 atoms of oxygen, then 1 mg. of adrenaline should require only ·022 c.c. of oxygen for its oxidation: since the amount of adrenaline added to the preparation was usually of the order of only ·02 mg., it is clear that no measurable error can be introduced by its oxidation by the oxygen present in the bath or respirometer.

Another objection might be that some drugs used have a specific effect on the tissue respiration, as cyanides for instance, which always depress it. This objection is also answered by the results obtained with adrenaline, which relaxes the cat's uterus (Exp. 11) and the intestine

content of the bath fall too low, and, if oxygen deficit is suspected, to run one period for a longer time than the others, in order to see whether it shows a diminished oxygen usage in unit time. In period 4 of Exp. 2, for instance, there was no falling off in the oxygen rate although the period was half as long again as the preceding ones; the oxygen supply was therefore adequate for a period of this duration, although a calculation shows that the oxygen-pressure during the period fell from 610 mm. Hg to 355 mm. Another factor which greatly affects the accessibility of the tissue for oxygen, is the size of the piece of tissue, as has been shown by Krogh for skeletal muscle (13): for this reason large masses of tissue were always avoided, and long thin organs, such as the guinea-pig uterus, or strips of intestine were found to give the best results.

The results obtained are best exhibited in a table, and Table I gives such a summary of experiments with tissue from various sources, both in the tonic and relaxed conditions, while Exp. 3 gives further details of an experiment in which the state of tonus was altered by the addition of the drugs (bath method), and Fig. 3 shows parts of the record obtained in this experiment.

Exp. 3. Rabbit uterus. 1·89 grams.

Period	Duration, mins.	Contents of bath	State of tone	c.c. O_2 per grm. per hr.
1	20	Ringer	Normal	·30
2	20	"	"	·29
3	20	" + ·2 c.c. 1 : 5000 adrenaline	Increased	·25
4	20	"	Normal	·26
5	30	"	"	·31
6	20	" + ·1 mg. histamine	Increased	·23
7	20	" + n/10,000 NaCN	Diminished	·22

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7	Rabbit vagina	—	·26	·25 (adrenaline)	"
8	" intestine	·79 (atropine)	·88	·68 (pilocarpine)	"
9	Guinea-pig intestine	—	1·43	1·11 (acetylcholine)	Micro-respirometer
10	Cat " (muscle only)	·18 (adrenaline)	·15	·13 (pilocarpine)	Bath
11	Cat uterus	·79 (")	·35	·12 (histamine)	"
12	Rabbit uterus	—	·29	·25 (adrenaline)	"
13	Guinea-pig uterus	—	·43	·38 (histamine)	Micro-respirometer
14	" "	·29 (adrenaline)	·36	·30 (acetylcholine)	"
15	Rabbit uterus	—	·29	·23 (histamine)	Bath
		Mean	·46	·39	

The results show that in the tonic state not more, but less oxygen is consumed, than by the same tissue when in the relaxed condition.

The effect of the act of contraction on the muscle has not been specifically investigated, but there seems no doubt that it involves, or is followed by, an increased oxygen consumption. This, I believe, explains the higher oxygen usages of the small intestine, which is in rapid rhythmic contraction, as compared with the more slowly-contracting tissue of the uterus (Figs. 3 and 4). Moreover, on investigating the oxygen usage of pieces of intestine from different parts, it is found that they are not equal, but show a gradation of oxygen usage corresponding approximately to the gradation of the rate of their rhythmic contractions. This result (though it has its exceptions) agrees in general with the view of Alvarez(16) that the metabolic gradient of the small intestine is from the duodenum to the ileum. The following figures illustrate the kind of results which are obtained.

Exp. 17. Pieces of small intestine muscle from different parts of the cat's bowel. Micro-respirometer method.

No. 1 from the duodenum. Oxygen usage = ·43 c.c. grm./hr.
No. 2 from middle of jejunum = ·31 „ „
No. 3 from lower ileum. Oxygen usage = ·288 „ „

The effect of oxygen lack. Although it would be premature and possibly erroneous to suppose that the mechanism of the contraction of plain muscle is very similar to that of skeletal muscle, there can be no doubt that in the absence of oxygen there is an accumulation within the tissue of products which are removed to some extent by immersing the tissue in Ringer's solution, and to some extent by oxidative processes. It is not surprising, therefore, to find that, as in Exp. 18, the oxygen usage of the tissue is enhanced by a previous period of oxygen lack.

Exp. 18. Large guinea-pig uterus. 2·1 grams. Bath method.

Period	Duration	Condition	c.c. O_2 per grm./hr.
1	30 mins.	Normal	·11
2	20 „	„	·12
3	120 „	In O_2-free Ringer	—
4	20 „	Returned to O_2 Ringer	·15
5	30 „	Normal	·21

As regards the nature of the accumulated materials thus removed by oxidation, and by washing, there is evidence, which will form the subject of a future paper, that lactic acid is at all events one of them.

The effect of temperature. The influence of temperature on the tissue respiration was examined in two experiments on the guinea-pig uterus, and the results are given in Fig. 5. For plotting this curve, the oxygen usages determined in one of the experiments were adjusted, from the usage at 37°, which was common to both experiments, so as to be comparable with the results of the other experiment. Interest attaches to

the temperature curve from two standpoints. It is seen from the figure that the oxygen usage increases, first slowly, then more rapidly, then

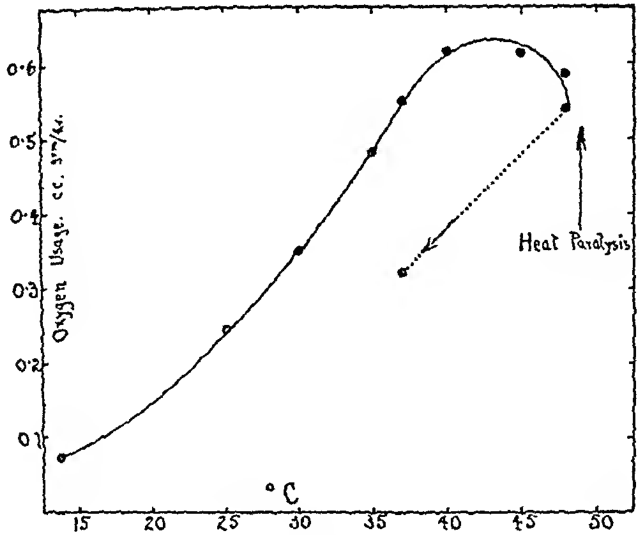

Fig. 5.

again slowly, until a maximum is reached at about 43° C., *beyond which* the value quickly falls off, and the tissue suffers permanent damage, as shown by the failure to recover when re-cooled (dotted line in figure). The phenomenon of heat paralysis, as I have previously shown (17) occurs at a temperature of 49° C. in this tissue, that is to say, at a temperature at which the oxygen consumption is still high, though apparently inadequate for the removal of the rapidly-accumulating products of metabolism. The general effects of temperature are similar to those seen on the heart (Evans (18)).

It is usual, in describing experiments on the effect of temperature on physiological phenomena, to discuss the relation of the results to the temperature coefficient of chemical reactions. Briefly, there is, as shown by Krogh (19), no constant relation between them, for when the values of the temperature coefficient are determined, they are seen to be quite inconstant. The values for Q_{10} were: from 15–25° C. = 2·9; from 25–35° C. = 2; from 35–45° C. = 1·3. This falling off in the temperature coefficient is what has invariably been found for other physiological processes, and is in no way surprising in view of the fact that the oxygen usage is only one aspect of the chemical changes in living tissues, and is undoubtedly influenced by a variety of physical factors, such as diffusion, viscosity, etc.

The effect of cyanide. In view of the fact (Evans(20)) that the action of cyanides on plain muscle resembles that of lack of oxygen, and of the well-known effect of cyanides in checking the oxygen usage of tissues generally, I have studied the effects of small additions of sodium cyanide to plain muscle. The following table gives the results in five such experiments with different concentrations of cyanide.

TABLE II.

Exp.	Concentration of cyanide	c.c. oxygen grm./min. Normal	After cyanide	% reduction
10	*n*/300	·40	·33	28
20	*n*/10,000	·31	·22	29
21	*n*/1860	·13	·065	50
22	*n*/930	·13	·056	57
23	*n*/10	·31	·062	80

When these results, with the exception of the first, are plotted, a curve resembling a hyperbola is obtained, and it would appear that the effects of the more dilute cyanide solutions are relatively much greater than those of the more concentrated ones. A somewhat similar insensitiveness to higher concentrations of cyanide has been observed by Lund(21), while Hartree and Hill(22) have also found that the delayed heat production of the frog's sartorius is by no means abolished by cyanides.

Taken in conjunction with the experiments of Lipschitz(23) on the reduction by muscle of *m*-dinitrobenzene, which is also not fully inhibited by cyanides, these experiments suggest that there are two kinds of respiratory process in plain muscle, one of which is inhibited by cyanide, while the other is not. The recent researches of Warburg(24) would suggest that the process which is inhibited by cyanide is one in which there is a metallic catalyst involved.

SUMMARY.

1. The oxygen usage of plain muscle was determined by two methods.
2. When in tonus the muscle uses less oxygen than when relaxed: this is because the surface of the fibres is reduced. The results of Parnas and Bethe, that no more oxygen is used, are thus confirmed.
3. After a period of oxygen lack, the oxygen consumption is increased.
4. Temperature increase, within physiological limits, increases the oxygen usage; the maximum is reached at 43°. The temperature coefficients at different ranges of temperature are not the same, but as in other instances show a diminution with rise of temperature.

5. Cyanides depress, but apparently do not abolish the oxygen usage of the tissue.

Part of the expenses of these experiments were defrayed out of a grant by the Government Grants Committee of the Royal Society.

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DOES INSULIN INFLUENCE THE GLYCOGENIC FUNCTION OF THE PERFUSED LIVER OF THE TURTLE? By E. C. NOBLE and J. J. R. MACLEOD.

(From the Physiological Laboratory, the University of Toronto.)

The remarkable effect which insulin has in causing glycogen to be deposited in the liver of completely diabetic (depancreated) dogs fed richly on carbohydrates would seem at first sight to indicate that a part at least of the sugar which disappears from the blood of normal animals following insulin injections is also due to glycogen formation. This might occur either in the liver or the muscles. But such a conclusion is not justified in the absence of direct evidence that glycogen is formed in the normal animal under these conditions. There are indeed certain theoretical reasons for assuming that such may not be the case. Thus, in the normal animal there may be available at all times a sufficient amount of insulin to convert into glycogen whatever glucose is not required for oxidation purposes so that the addition of more insulin, as by its injection, cannot cause any more glycogen to be deposited. Obviously, this state of affairs is vastly different from those obtaining in a depancreated animal in which glycogen formation is impossible because of the absence of insulin. To obtain direct evidence whether or not insulin causes increased glycogen formation in normal animals two types of experiments that have been employed are perfusion of the turtle liver with artificial plasma and comparison of the sugar content of the blood of the portal vein and vena cava in anæsthetised dogs. The present paper gives an account of the observations on the turtle liver.

Grube (1) was the first to call attention to the possibility of using the turtle liver for the study of glycogen formation, because of the fact that its main blood supply is carried to each of its two lobes by separate branches of the umbilical vein. In his earlier experiments he removed one lobe after tying a ligature around the isthmus which joins the two lobes, and then perfused the remaining lobe with Ringer's solution containing the substance whose influence on glycogen formation he desired to investigate. The glycogen content of the two lobes was then compared. This method was afterwards abandoned because the extensive glycogenolysis occurring in the perfused lobe served to mask any glycogenesis

that might have taken place. The later experiments consisted in perfusing each lobe separately and adding to the fluid on one side, the substance to be investigated. Under these conditions glycogenolysis would presumably proceed at an equal rate in both lobes except in so far as it was influenced by the added substance. Even this refinement does not make the method an ideal one, because, as shown by Schöndorff and Grebe(2), there are normally considerable differences in the glycogen content of the two lobes (amounting often to 32 p.c.), the left lobe usually containing the larger amount. Despite these sources of error there is no doubt that glucose can be deposited as glycogen in the turtle liver perfused outside the body with Ringer's solution containing glucose (cf. also(3)) and it seemed worth while to see whether the addition of insulin to the perfusion fluid might accentuate the process. The negative character of the results obtained by this method led us to employ that described by Snyder, Martin and Levin(4), namely to observe the amount of sugar in the fluid perfused through the liver in a unit of time having regard also to the *p*H of the fluid. These authors by careful analysis of the effects produced by variations in *p*H, with or without the presence of minimal quantities of epinephrin, have shown that the sugar output depends mainly upon the minute volume-flow through the liver. When this is increased, as by lowering *p*H (making the solution more acid), two factors may act to increase the sugar output, first more of the liver is irrigated by the perfusion fluid so that more cells come into action, and second, the diastatic enzyme (glycogenase) in the cells becomes more active since chloride phosphate solutions augment the diastatic activity at low *p*H more than at high *p*H (Langfeldt). These authors are inclined to believe that the supposed direct influence of epinephrin on the glycogenolytic process is dependent upon the changes in minute volume-flow which it causes and the nature of which will vary with the *p*H of the perfusion fluid.

Methods. Most of the experiments in which glycogen deposition was observed were performed by perfusing each lobe of the liver separately with the same artificial plasma after tying a mass ligature around the isthmus joining the two lobes. To the fluid of one side, insulin was added and after perfusion for several hours the glycogen in each lobe was determined by Pflüger's method. In some of the experiments only one lobe was perfused, the other having been removed at the start. In all these experiments an outflow cannula was inserted in the aorta and the fluid was perfused at constant pressure through the liver (which was left *in situ*).

For the experiments in which the minute output of sugar was observed, greater precautions had to be taken. While the liver was still *in situ* the numerous small branches of the portal system running between the liver and the stomach and duodenum were ligated (*G.V.*, Fig. 1).

In one or two of the experiments it was found that there was great irregularity in the perfusion of the excised liver, when the perfusion fluid entered only by the two umbilical veins. Consequently a third cannula was inserted in the large spermatic vein (which by its large size, and its effect upon the perfusion, might indeed seem to convey a large part of the blood to the organ)—see Fig. 1. By this means it was sought to rule out the considerable variations which were found to occur in the per cent. of sugar in the perfusion fluid due to some of the fluid from time to time finding its way into a non-irrigated portion, and carrying out with it some of the sugar produced by post-mortem glycogenolysis.

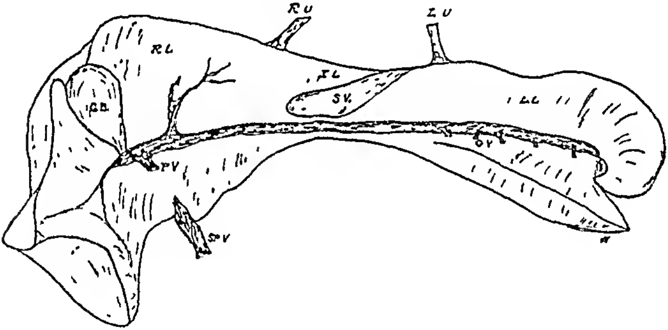

Fig. 1.

The liver was excised, with the heart attached, and placed in a large Buchner funnel; it was kept moist by a wet filter paper lid. Care was taken to prevent any disturbing of the preparation, as this, and also variations in the pressure of the perfusion fluid, were found to cause fluctuations, more or less marked, in the sugar output. Oxygen was continuously bubbled through the perfusion fluid.

The perfused fluid was collected in graduates and the outflow per unit of time measured. In some of the experiments the fluid after the removal of samples for analysis was replaced in the perfusion bottle. In others the perfusates were not used over again. The pH was measured,

after dialysis, by the colorimetric method and the sugar, by the Shaffer-Hartman method. Usually the liver was markedly œdematous at the end of the observation which lasted 4–6 hours. This made it impossible to determine the glycogen content with any degree of accuracy.

Controls on the glycogen content. The glycogen content of the two lobes was determined:

(*a*) The entire liver was removed from three turtles after stunning and the glycogen in each lobe determined immediately.

Glycogen (p.c.) Right lobe	Glycogen (p.c.) Left lobe	Percentile difference (greater in right lobe)
6·63	5·66	14·6
7·59	6·77	10·8
4·92	4·75	3·4

(*b*) The left lobe was removed after tying a ligature around the isthmus and the glycogen determined immediately. The right lobe was perfused through the umbilical vein with artificial plasma (Locke's solution) usually containing less than ·1 p.c. of glucose.

Glycogen (p.c.) Right lobe	Glycogen (p.c.) Left lobe	Percentile difference (greater in right)	Duration of perfusion hours	P.c. glucose in perfusate
7·60	4·50	40·5	5	·038
6·35	5·44	14·3	7	None
3·14	2·26	28	4	Unknown
5·75	4·67	18·8	3	·35
3·77	4·41	14·5 (greater in left)	5	·075

It is seen that there was decidedly more glycogen in the right lobe in seven out of the eight observations. Without perfusion this difference varied between 3·4 and 14·6 p.c. and when the right lobe was perfused with solution containing glucose the difference became decidedly greater (up to 40·5 p.c. in one case). In one observation, however, there was less glycogen after perfusion. The results confirm those of Grube, Nishi, etc., in showing that glucose can cause glycogen to be formed but the extent to which this occurs is variable in different livers and sometimes instead of an increase a decrease is observed. In the unperfused liver we have found the larger percentage of glycogen in the right lobe.

The effect of adding insulin to the fluid perfused through one of the lobes. Both lobes were perfused with Locke's solution. The insulin used contained no preservative (trikresol) and its potency was determined by the rabbit test. It was faintly acid in reaction and (in numbers 1–5) equal portions were added to the fluid perfused through both lobes, one of

these portions being boiled under the mistaken impression that this would diminish its potency. In numbers 6–10 active insulin was only added to one bottle. Sometimes the active insulin was perfused through the right lobe, sometimes through the left. The following table gives the results, the side perfused with active insulin being indicated by an asterisk

No.	Glycogen (p.c.) Right lobe	Left lobe	Percentile difference (greater in right)	Duration of perfusion, hours	P.c. glucose	Remarks
1	4·71*	4·50	3·1	3½	·5	Insulin inactive
2	9·21	8·37*	9·1	3	5	,, active
3	7·04*	0·13	13	3	5	,, ,,
4	8·37	8·23*	1·6	4¼	·25	,, ,,
5	4·02*	8·02	—	3	25	,, ,,
0	1·65	1·28*	1·8	7¼	5	,, ,, (skate)
7	2·72	2·54*	7	7½	5	,, ,, (,,)
8	·97	·02	36	7½	5	,, ,, (,,)
9	3·42	3·20*	6·4	9¾	1·0	,, ,, (,,)
10	1·84*	1·43	22·3	10	2	,, ,, (,,)

It is clear that the results are similar to those of normal livers There are evident fallacies involved in the method on account of the irregular distribution of glycogen between the lobes Moreover, it is doubtful whether insulin acts with anything like the same promptitude in cold blooded, as in warm blooded animals. We observed, for example, that enormous quantities of insulin can be injected into the dorsal lymph sac of frogs without any symptoms of hypoglycæmia being produced up to a period of four days. Krogh (5) has, however, found that hypoglycæmic symptoms develop later. We have also observed the effect of insulin on the percentage of sugar in the blood of turtles injected with insulin with the following results (in all cases the blood was removed from the heart through a trephine hole in the shell):

11.35 a.m. Blood from heart ·030 p.c. glucose
11.30 a.m. 1 c.c. active insulin injected into heart
3.55 p.m. Blood from heart ·125 p.c. glucose

11.54 a.m. Blood from heart ·063 p.c. glucose
1.00 p.m. Turtle given ether
1.01 p.m. Blood from heart ·112 ,, ,,
3.35 p.m. Blood from heart ·134 ,, ,,

11.51 a.m. Blood from heart ·067 p.c. glucose.
11.55 a.m. 2 c.c. active insulin injected subcutan. into leg
2.00 p.m. Blood from heart ·095 p.c. glucose

(4) 12.10 p.m. Blood from heart ·063 p.c. g
2.35 p.m. Blood from heart ·117 ,,

(5) 12.00 noon Blood from heart ·031 p.c. g
12.10 p.m. 2 c.c. active extract injected
3.25 p.m. Blood from heart ·047 p.c. g
5.10 p.m. Blood from heart ·037 ,,

(6) 12.30 p.m. Blood from heart ·051 p.c. g
(considerable hæmorrhage)
3.20 p.m. Blood from heart ·086 ,,
5.15 p.m. Blood from heart ·143 ,,

In every case a decided increase in blood sugar occurred and insulin did not have any perceptible influence. The increase is probably due to the relatively large loss of blood involved in removing 1 c.c. (the average weight of the turtles was 900 gms.). In some cases it was due to ether. Since it has been shown that insulin can prevent the hyperglycæmia due

to asphyxia and ether in rabbits, it seems reasonable to conclude that it does not cause a reduction in the percentage of blood sugar that is at all comparable with its effect on warm blooded animals. It may be for this reason that we have not been able to demonstrate any influence of insulin on the glycogen function of the turtle liver.

The effect of insulin on the percentage of sugar in Ringer's solution re-perfused through the liver. In all cases the perfusion fluid contained $M/30$–$M/60$ phosphate solution and the pH was frequently tested during the perfusion. Although the reaction of all perfusates was adjusted to a pH of about 7·35 to start with, it was noted that they became usually more alkaline during the perfusion, the pH rising to 7·7 or even to over 8 (as in Fig. 2).

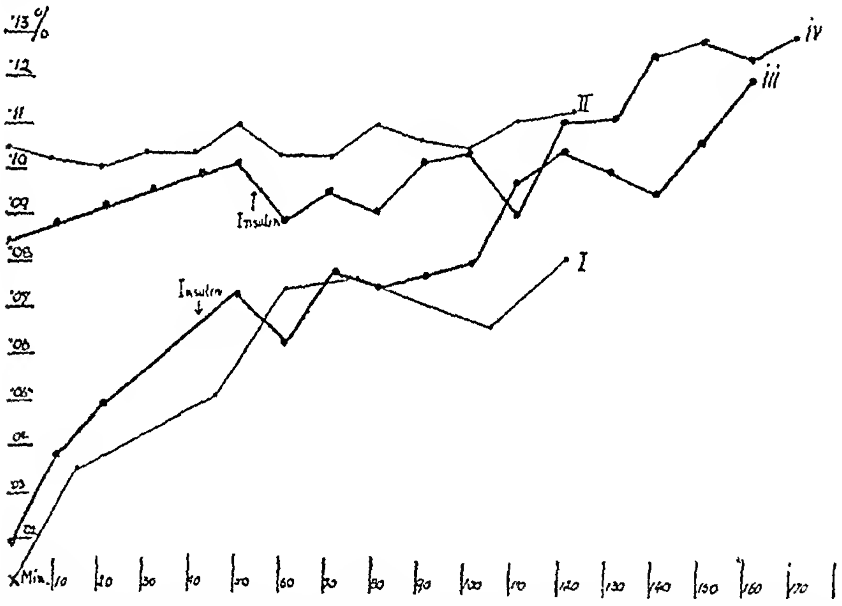

Fig. 2. The percentage of sugar in artificial plasma re-perfused through the liver of the turtle. Insulin was added to the fluid in the two experiments recorded by the thick line curves. pH of the perfusates: I, 8·2; II, 7·2–7·7; III, 7·3–7·7; IV, 7·35–7·7. Ordinates, p.c. sugar. Abscissæ, time in minutes.

The results are shown in the curves of Fig. 2, in which the ordinates give the percentages of sugar in the perfusion fluid and the abscissæ the time intervals after the start of the observation. In two of the experiments, viz. I and II, no insulin was added to the perfusion fluid. One of them (II) showed a very small increase in sugar concentration indicating that very little glycogenolysis could have been occurring. In the other (I), however, the concentration increased considerably. The more

rapid glycogenolysis appeared to occur in the perfusate which was the more alkaline. In the two other experiments of this group (III and IV), insulin was added to the perfusate. In III it was added, two drops at a time every 10 minutes beginning 40 minutes after the start of the observation, and it will be observed that it did not alter the average rate at which the concentration of sugar rose in the perfusion fluid. Since the perfusion fluid in this experiment contained ·02 p.c. sugar at the beginning and ·112 p.c. at the end of the perfusion, it is clear that considerable glycogenolysis was occurring, the rate of which insulin apparently did not affect. A part of the increasing percentage of sugar in the perfusate is to be accounted for by reduction in volume, due to the removal of samples for analysis. If we allow for this there may be a slight falling off in the rate of glycogenolysis during the insulin period, but it is insignificant. In the other experiment of this group (IV) the insulin, after adjusting its *p*H to that of the perfusate, was added (2 c.c.) between 50 and 60 mins. after the start, as indicated by the arrow. After a temporary decrease due in part to the dilution, the increase in sugar concentration proceeded at the same average rate, with the exception of one estimation (at 110 mins.).

These experiments indicate that insulin has no influence in diminishing the rate of post-mortem glycogenolysis naturally occurring in the perfused turtle liver.

The effect of insulin on the sugar output in equal periods of time when Ringer's solution is perfused once through the liver. The liver was removed from the body, after inserting the necessary cannulæ and the perfusate collected during each 10 minutes was measured and the amount of sugar determined. The results are given in Fig. 3, in which the ordinates are obtained by adding the amounts of glucose appearing in the perfusates collected during each 10-minute period. The perfusate in all experiments contained a sufficient amount of a mixture of phosphates to hold the H-ion concentration fairly constant. The rate of perfusion was kept as uniform as possible for it was found that any increase in this caused more sugar to appear in the perfusate. The average perfusion rate in c.c. per 10 mins. is indicated in the curves by the figures marked *P.R.* Three such experiments (6, 7 and 8) were done without adding insulin to the perfusate and three (2, 4 and 5) in which it was added in various amounts.

In the three experiments with insulin several 10-minute portions of perfusate were collected prior to adding insulin and the normal rate of sugar output for that liver determined. It will be seen that this was similar in two of the experiments (3 and 4) and decidedly less in the other

(5) and that it bore no relation to the perfusion rate. The pH of 2 was 7·3 and of 5, 7·0, and in both cases this remained constant throughout the experiment. The pH of 4 was 7·4 to start, but it varied somewhat during the observation, falling at one stage to 7·15. Varying quantities of insulin were used: in 2, 8 c.c. of an active preparation in 75 c.c. of perfusate added between 30 and 80 minutes; in 4, 1·5 c.c. were added to 200 c.c. perfusate between 30 and 90 minutes; and in 5, 2·5 c.c. were added to 200 c.c. between 30 and 100 minutes.

It will be seen that these varying concentrations of insulin in the perfusate did not influence the rate of sugar output from the liver, neither immediately nor after a period of 90 minutes. The conclusion is that the rate of post-mortem glycogenolysis in excised turtle liver is not influenced by the presence of varying concentrations of insulin at ranges of pH between 7·0 and 7·4.

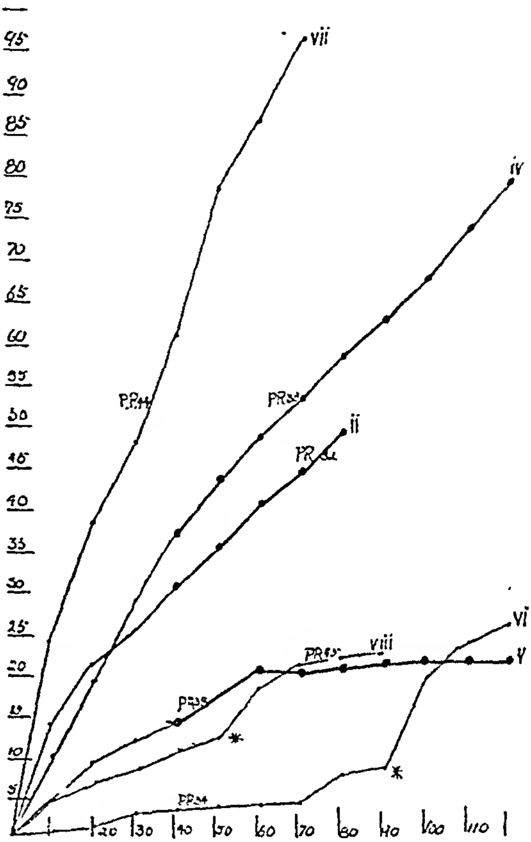

Fig. 3. The percentage of sugar in artificial plasma perfused once through the liver of the turtle. The curves show the amounts of sugar found in the fluid collected during each 10-minute period of perfusion, the amount for each period being superadded. pH of the perfusates: VI, 7·1–7·2; VII, 7·1–7·2; VIII, 7·3–7·4; II, 7·3; IV, 7·15–7·6; V, 6·9–7·15. Ordinates, p.c. sugar. Abscissæ, time in minutes. *P.R.* indicates the rate of perfusion in c.c. per 10 minutes. Insulin was added to the perfusion fluid during the periods indicated by the thick line curves.

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THE RATE OF RECOVERY OF NERVES IN ASPHYXIA.

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WORK on the behaviour of nerves in asphyxia was carried out by Verworn and his co-workers at the beginning of the present century; he argued that since nerve cells and other living substance were fatigued in absence of oxygen, there was good reason to suppose that nerves behaved in a similar way. Von Baeyer(1) found on exposing a stretch of frog's sciatic nerve to nitrogen or hydrogen and stimulating electrically either on the stretch or central to it, that the nerve ceased to be excitable in 3–5 hours; on replacing the asphyxiating gas by air, there was very rapid recovery in 3–10 minutes. Fillié(2) repeated these experiments, using oxygen free saline solution, and obtained the same results. He worked out the minimum value of oxygen necessary to maintain conduction and found this to be between ·1 and ·3 mg. oxygen per litre, or about ·03 p.c. by volume. Further work was carried out by Fröhlich(3)(4), he postulated a region of decrement in the stretch of nerve asphyxiated, this was shown by a reduction of the propagation velocity of the impulse. He attempted to explain the mechanism whereby the nerve uses the oxygen and stated that asphyxia hindered the assimilation of oxygen and that the nerve contained a reserve store which it could only call upon if the pressure conditions were correct. Normally the oxygen maintained the excitability constant and delayed the appearance of asphyxia. He also studied the effect of the condition of the frog at the time of the experiment and stated that this affected the results both during and in the recovery after asphyxia.

At this time the excitability was expressed by the value for the threshold of the electrical stimulus on the asphyxiated stretch, and the conductivity was measured by the somewhat crude method of noticing if there was any muscular contraction on stimulating the nerve central to the asphyxiated part. Experiments showed that during asphyxia there was a gradual fall of excitability, and a sudden fall of conductivity just before the impulses failed to get through to the muscle. In order to obtain a more detailed account of the happenings in the nerve, Fröhlich(5) studied the change of refractory period. He sent in a series

of faradic stimuli at a known period and found at what period the muscle ceased to respond with a complete tetanus. He maintains that in asphyxia the value for the refractory period rises from the normal value of ·003″ to over ·1″ and from this he concludes that the nerve is unable to recover at the same rate as before. The only other record of a nerve having a very long refractory period is in the case of the application of yohimbine investigated by Tait and Gunn (6); they found an absolute refractory period rising to ·04 and a total period of 2·2 secs. It seems very probable in this case that the effect is specific in nature, and with such a long refractory period it is reasonable to suppose that the rate of recovery is also affected.

Lucas (7) studied the effect of alcohol on the rate of recovery of a nerve and incidentally elucidated several important facts concerning the behaviour of the nerve in narcosis. He showed clearly the distinction between the decrement in the nerve involving impaired conduction, and the recovery of the nerve after the impulse had passed. And with the help of some of Adrian's (8) work he makes it quite clear that the normal recovery of a nerve is expressed by the curve relating the interval for muscular summation to the strength of the second stimulus, the curve *AB* of Fig. 1. And that when the nerve is subjected to the action of a

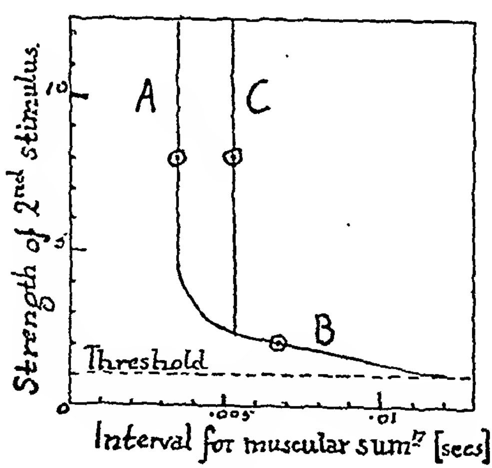

Fig. 1.

narcotic the curve takes the form *CB* in which the vertical part *C* is an expression of the decrement in the nerve, and *B* is still part of the original recovery curve and expresses the rate of recovery. By taking measurements of the least interval for muscular summation

during the passage of alcohol using a second stimulus of eight times the strength, *i.e.* determining a point on the steady part of the curve where the least interval does not decrease for an increase of the second stimulus, he is able to follow the onset and course of the decrement; the interval shows a gradual increase, whereas the "recovery time," the interval for muscular summation using a second stimulus of twice the threshold strength, remains steady until it is equalled by the least interval. From this, and other confirmatory experiments, he concludes that the conduction in a nerve and the rate of recovery are quite separate processes and that the latter is unaffected by narcosis. Since narcosis and asphyxia have been shown to have many similarities in their action on nerves, it seemed very possible that the unaltered rate of recovery would be found on asphyxiating a nerve with an inactive gas.

The experiments were carried out on the sciatic-gastrocnemius preparation of a frog, and an ebonite muscle nerve trough was used (Fig. 2).

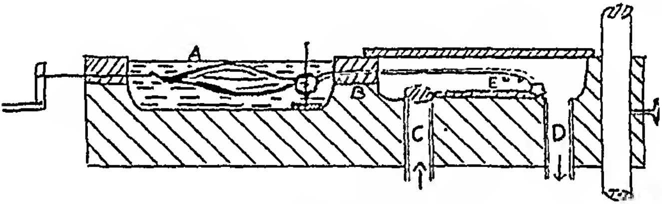

Fig. 2.

The muscle was placed in Ringer solution in the chamber *A* with its free end attached to a writing lever and the nerve passing back through a slot *B*, plugged with vaseline, into the nerve chamber where it passed over a pair of platinum electrodes *E* about 2·4 cms. from the slot. The nerve chamber had an inlet tube *C* and an exit tube *D* and it could be closed by a glass plate sealed on the top with vaseline. It was found advisable to have some blotting paper soaked in Ringer solution on the floor of the nerve chamber and also a small damp wad of cotton wool placed loosely in the opening of the inlet tube. At one time a second pair of electrodes were placed at about 4 mm. from the slot *B*, but these had to be abandoned as there was invariably current spread. The inlet tube could be connected with either a Mariotte bottle forcing damp air through the chamber or else with the source of hydrogen gas used for asphyxiating; the exit tube led to a wash bottle containing water, this served to prevent the entrance of air, and also on comparing the number of bubbles of gas

passing through a wash bottle just before entering the chamber with those issuing through this final bottle, it was possible to see whether the chamber had any appreciable leak. The hydrogen was obtained from a cylinder provided with a fine adjustment release cock so that the stream could be regulated. It passed through two absorption tubes containing alkaline pyrogallol and then through a wash bottle containing water; the resulting gas proved on analysis to contain about ·025 p.c. oxygen, an amount just lower than the one postulated by Fillié. The stimuli were break shocks from two coreless induction coils, the first coil was adjusted by moving the secondary coil relative to the primary, and the second had an adjustable resistance box in the primary circuit. The primaries of both circuits were opened by a spring contact breaker with two adjustable keys which could be set so that one was opened at a known time interval after the other.

When the preparation was set up, a slow current of air was allowed to flow until the threshold, the least interval for muscular summation and the recovery time were steady. This usually took about an hour an a half, and the values reached by that time were found in a control experiment to be maintained for five hours or more. Before turning on the hydrogen a recovery curve was plotted, for this is a good record of the state of the nerve. The air was then turned off and a current of hydrogen started; a stream of about 30 bubbles a minute was used. The nerves maintained their excitability and power of conduction in hydrogen for a period varying from three to five hours, agreeing in this detail with the results of von Baeyer and others. But during this time the least interval for muscular summation increased showing the onset of conduction with a decrement. Fig. 3 shows a typical set of results.

The upper curve shows the alteration of the threshold as the percentage of the normal value, the curve remains steady or rises slowly during the early stages of asphyxia, then there is often a sharp rise just before the excitability fails completely. The lowest curve shows the course of the least interval for muscular summation, this too remains steady for some time, then on the rise of the threshold it also begins to increase in value as the decrement becomes more pronounced. This rise may occur before or after the rise of the threshold, the experiments showed no majority one way or the other. The sharp final rise usually took about an hour, at the end of this time the nerve was no longer excitable and the least interval had risen to a mean value of about ·012 sec. a value comparable with the length of the total refractory period and in no-wise suggesting the value of ·1 sec. put forward by Fröhlich as the limit

reached. The middle curve shows the recovery time of the nerve, the values found remained approximately the same throughout the course

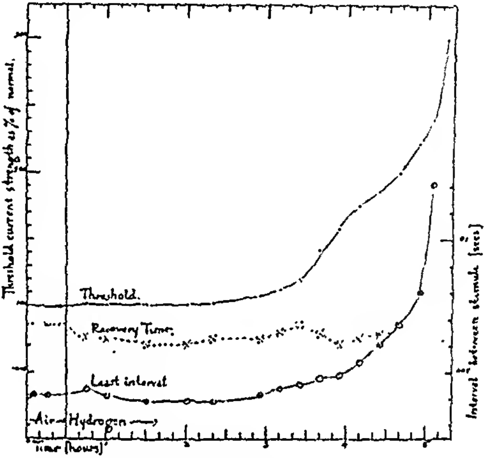

Fig. 3.

of the experiment until the value for the least interval equalled them. Sometimes the recovery time showed slight fluctuations in value when the least interval was beginning to rise, but these were never very great and can possibly be explained by the experimental difficulty of obtaining an accurate value for the threshold; when the latter is increasing rapidly the strength of the second stimulus might be less than twice the threshold and the value for the recovery time would be slightly too large.

Expt.	Asphyxia time	Limits of recovery time	% rise	Limits of least int.	% rise
1	5h 0m	·0066 to ·0079	120	·0035 to ·0121	340
2	3 0	·0078 ·0088	113	·004 ·0132	330
3	4 50	·0061 ·0065	106	·0039 ·0122	287
4	3 0	·0073	100	·0040 ·0121	220
5	3 50	·007 ·008	114	·0035 ·014	400

In the table the percentage rise of the recovery time shows an average value of 110 whereas the least interval for muscular summation rises to about 320 p.c. of its resting value, these figures are in no-wise comparable with each other and one may conclude that the recovery time is unaltered.

After the asphyxia it is of interest to note that the nerve did not

and conductivity; but it needs it to oxidise the break down products formed during the passage of the impulse, so that the next impulse may find an adequate local supply of energy for its transmission.

I am indebted to Mr J. B. S. Haldane for the analysis of the hydrogen, and I wish to thank Dr E. D. Adrian for his kindly criticism and advice throughout the work.

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ANTIDROMIC ACTION. Part II. Stimulation of the peripheral nerves of the cat's hind foot. By J. N. LANGLEY.

(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

THE general conclusions arrived at in Part I (1) were that antidromic nerve impulses cause dilatation of the capillaries of the foot of the cat but not of the arteries from the iliac artery down to the branches given off by the digital arteries, and probably that antidromic impulses do not directly cause dilatation in the final arterial branches. It seemed that further light on the subject might be obtained by stimulating the several peripheral nerves.

It was found that stimulating the posterior roots of the 7th lumbar nerve after section of one of the branches of the plantar nerves, commonly caused pallor in the area which did not flush. Since stimulation of a plantar nerve branch caused similar pallor in the non-flushing region, experiments on the peripheral nerves afford a convenient method of determining the cause of the pallor.

From these points of view the experiments recorded in this Paper were undertaken. The cats were anæsthetised in the manner described in Part I and lay unbound on a warmed table.

1. *The effects produced by stimulating the peripheral nerves.*

The superficial branches of the plantar nerves were stimulated a little proximally of the pad. The anatomical course of these nerves has been described in Part I. It may be recalled that they are sensory only and that the branches are distributed as in Table I.

TABLE I.

Internal plantar	Medial branch to medial side of 2nd toe
"	[illegible]
"	
External plantar	
"	Lateral branch to lateral side of 5th toe

We may give first the effect on secretion of sweat, since this is usually the most local. Each branch causes secretion in the anatomical area just given. When it runs to one side only of a toe, the secretion is never on the whole surface of the cushion. Sometimes it is strictly confined to one longitudinal half, but not infrequently a few drops occur 0·5 to 1 mm. over the mid line, and more frequently in the proximal part and the tip than in the mid region. Similar unilateral secretion occurs on stimulating each plantar digital nerve. In some cats, as is known, secretion is scanty. In these, secretion occurs either in part of the longitudinal half of the cushion, or in the whole of it only after repeated stimulation. Thus there is but a trifling overlap in the peripheral distribution of the secretory fibres of the plantar digital nerves, and as secretory activity decreases no overlap is observable. The overlap of secretion from side pad to mid pad and from mid pad to side pad varies but is not more than 2 mm.

The effect on the colour of the foot of the vaso-constrictor fibres of the superficial plantar nerves is usually overpowered by antidromic vaso-dilator impulses. The first stimulation may cause either pallor or flushing, and usually a weak first stimulation—one just felt on the tongue —causes pallor, and a strong stimulus causes flushing. If pallor is caused by the first strong stimulation it gives way to flushing as the stimulus is kept on, and after a few repetitions of the stimulus there is flushing only. Occasionally there is pallor in one part of the area anatomically supplied by the nerve branch and flushing in another part, but in all cases obvious flushing, and in most cases great flushing, is obtained either with the first or with later stimulations.

Whilst stimulating any one of the superficial plantar nerve branches will at some time or other cause flushing in the area of distribution of the branch as given in Table I, the flushing may be more extensive in the cushion of the toe than the distribution there given, and it may even occur on the whole of it, although the nerve can only be traced to one side. Similarly on stimulating any one plantar digital nerve, the flushing may be fairly strictly homolateral in the cushion, or spread to a part or the whole of the opposite side. The chief condition affecting the outspread from one side to the other has been mentioned in Part I, viz. previous antidromic vaso-dilation on the opposite side, *i.e.* the state of tone of the vessels at the extreme periphery. The effect is readily seen by successive stimulation of the several plantar branches. Thus if the superficial external plantar nerve is stimulated, the flushing on the 4th toe is usually on the lateral side only, but if it is stimulated some minutes after stimulation of the internal plantar (or of its lateral branch) the flushing spreads

more or less into the medial side of the 4th toe and may include the whole of it.

Variation of tone of peripheral vessels does not however account for another variation in the degree of outspread of flushing from one side of a cushion to the opposite side. We have seen in Part I that each toe is supplied with a digital artery which runs along one side of a toe and crosses under the tendon of the flexor brevis to supply the opposite side. We may then speak of one side as the proximal artery side, and the other as the distal artery side. The two sides are innervated by the superficial plantar nerves as in Table II.

TABLE II.

Distal	artery	side of	2nd	toe	Int. pl. medial branch
Proximal	"	"	2nd	"	" central "
Distal	"	"	3rd	"	
Proximal	"	"	3rd	"	" lateral "
Proximal	"	"	4th	"	
Distal	"	"	4th	"	Ext. pl. medial "
Proximal	"	"	5th	"	
Distal	"	"	5th	"	" lateral "

When no plantar nerve has been stimulated, the usual effect of stimulating a branch which supplies the distal artery side of a toe is to cause flushing of the cushion fairly strictly confined to that side, and stimulating a branch which supplies the proximal artery side is to cause flushing spreading over the mid line chiefly at the proximal part and tip, but not extending to the whole of the opposite side. The part which does not flush as seen from the plantar surface is roughly an oval area (cp. Fig 1 *B*) and this becomes paler. It will be convenient to speak of this pale area as the "oval" area, though it is not usually curved on the dorsal surface.

After other nerves have been stimulated there is a variable degree of outspread not only from one side to the other, but in different parts of the cushion, and as I have said the whole of the cushion may flush when the nerve on either side is stimulated.

The customary greater outspread of flushing from proximal artery side to distal artery side cannot be due to dilatation of the proximal artery since the whole of the distal artery side does not flush. It might be due to a few nerve fibres from the proximal artery side crossing with the artery to the opposite side but I have not been able to trace such fibres, and if there were such, one would expect the middle of the opposite side to flush as much as the ends. In some cases, nerve stimulation on the proximal artery side causes secretion farther over the mid line in the area which flushes than does stimulation of nerve on the distal artery side,

suggesting a greater crossing of nerve fibres in the cushion itself, but I have not found this constant, and the chief causes of the greater outspread from flushing we are considering is I think the position of the veins.

In each cushion a small vein (efferent vein) can be seen starting $\frac{1}{3}$ to $\frac{1}{2}$ way from the tip, generally a little on the side of the distal artery. Its course is not along the mid line of the cushion, but to the side of the digit which has the distal part of the artery as in Fig. 1 *A*. Leaving the cushion it joins a plantar venous network.

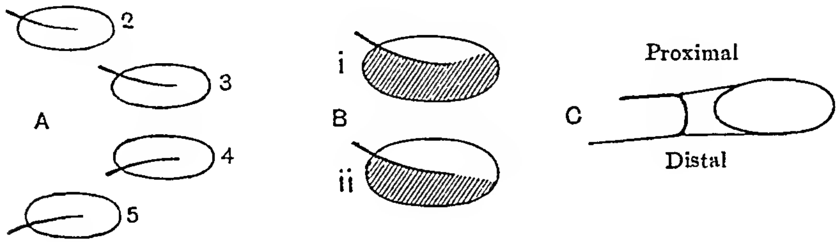


Fig 1

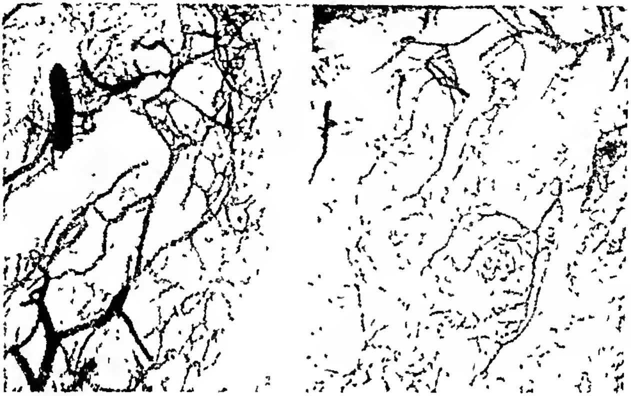
Fig. 1. *A*. Course of the plantar efferent vein from the dermal venous network in the several toes.

B. i Common extent of flushing (shaded) from proximal artery side to distal artery side. ii. Extent occurring less commonly.

C. To illustrate a relatively greater decrease of blood on the opposite side to that which flushes, when the flushing is on the distal artery side than when it is on the proximal artery side (cp. p. 55).

On stimulating the nerve supplying the proximal artery side the flushing can as a rule be seen to be bounded by the vein as far as the vein is visible (cp. Fig. 1 *B*). Obviously if there were a network of capillaries or veins under the epidermis, flushing on the proximal artery side would cause increase of pressure in the network up to the efferent vein and the greater outspread of flushing from the proximal artery side to the distal than from the distal to the proximal would be accounted for. In order to determine whether a network of capillaries or veins exists, I had injections made by my laboratory assistant Mr Freeman. Fig. 2 *a* is a photograph of an oblique section through the epidermis and derma, so that the vessels in the derma have approximately the appearance they have in sections parallel with the surface. The capillaries of the papillæ end in a loop and there is only a slight capillary connexion beneath the epidermis. In the derma there is a close network of small veins which receive blood on the one hand from the papillæ and on the

other hand from the superficial part of the subcutaneous tissue of fat with sweat glands. In the fat lobules there is a very close network of capillaries, smaller than those of the papillæ (Fig. 2 *b*). In specimens injected with nitrate of silver and stained with hæmatoxylin I have not



(*a*) (*b*)

Fig. 2. (*a*) Oblique section of lower part of epidermis and of the derma, showing dermal venous network and capillaries of papillæ (*b*) Two lobules of fat with sweat glands showing close network of capillaries, the superficial veins from these join the dermal venous network in the upper part of the figure. Magnification shown by the lines 10 μ apart.

found any indications of either a muscular coat in the venous plexus or of valves. The arrangement is essentially the same as that which has been described by Spalteholz(2) in the human skin. The existence of a dermal venous network with an asymmetric efferent vein accounts for the outspread of flushing being ordinarily greater from proximal artery to distal side, than in the contrary direction. Further, the more the tone of the vessels on the non-stimulated side is lessened the farther naturally the flushing will spread across, and so unilateral stimulation will cause bilateral flushing. But this I think implies that antidromic nerve impulses cause loss of tone in the dermal venous plexus as well as in the capillaries.

The histological results make it practically certain that antidromic

flushing in the foot is largely due to increased blood in the venous network. And this conclusion is in harmony with the prompt flushing produced by increased venous pressure and also with the slight flushing which (so far as I have found) occurs in the skin of the leg. In this the dermal venous plexus and the subcutaneous fat are comparatively little developed. Further, it is doubtful whether the degree of flushing which occurs can be accounted for by dilatation of the papillary capillaries plus the distension of the venous network it causes. It would seem that in the antidromic flushing of the foot there must also be either dilatation (loss of tone) of the venous network or distension of them by blood flowing from dilated capillaries of the subcutaneous tissue. In other words, that the antidromic vaso-dilation is not confined to the skin capillaries and I show in another paper that the sympathetic nerves can cause flushing in the foot by setting free metabolites in the sweat glands; thus on stimulating the plantar nerves the secretion has to be taken into account. Flushing and visible secretion are, however, independent changes. It is common to obtain flushing without secretion, and flushing is obtained, though it is usually lessened, after intravenous injection of 10–20 mgm. of atropine sulphate. Free secretion with slight flushing sometimes occurs towards the end of an experiment.

Cold causing local arterial contraction when combined with lower blood-pressure or with much loss of blood greatly reduces the effect of stimulating the plantar nerves. But flushing when established is apparently very little affected by cold, for in one experiment in which the posterior roots of the 7th lumbar nerve caused a brilliant flush, there was very little change on packing it round with ice though possibly the flush was a trace bluer. Dale and Richards(3) have pointed out that cold causes capillary congestion with arterial contraction.

Flushing in one part of the foot produced by stimulating a plantar nerve branch is accompanied normally by pallor in the rest of the foot, and the pallor is usually greater than that produced by stimulating the posterior roots of the 7th lumbar nerve after section of one of the plantar branches (cp. Part I). The pallor which occurs on the side of a toe opposite to a stimulated digital nerve has already been referred to. There are some other points to notice.

The degree of pallor varies greatly. In the course of an experiment the non-flushing toes may become dead white or there may be no certain change of tint. It is roughly proportional to the degree and area of flushing. Thus it is generally greater on stimulating the nerve branch which causes flushing of the mid pad than on stimulating either of the branches which

cause flushing of a side pad, and each of these causes more pallor than the branches which run to one side of a digit only.

On stimulating the digital nerve of one side, the pallor on the opposite side is generally greater when accompanying flushing of the distal artery side than when accompanying flushing of the proximal artery side. Taking the circulation from the point where the digital artery crosses to the opposite side of the toe (Fig. 1 *C*), dilatation on the distal artery side will take more blood from the branch to the cushion of the toe on the proximal artery side than dilatation on the proximal artery side will take from the branch to the cushion of the toe on the distal artery side. The nerve which causes flushing of the mid pad (cp. Table I) is the lateral branch of the internal plantar. Stimulation of this branch usually causes great pallor in the side pads, good pallor in the 2nd and 5th toes and appreciably less pallor in the "oval" areas of the 3rd and 4th toes.

In the 2nd and 5th toes the pallor begins and may be nearly confined to the sides facing the mid line of the foot, indicating that the other sides receive some blood from the saphenous arteries. Further, the 2nd toe not infrequently becomes paler than the 5th. Ligature of the digital artery and nerves of the 3rd or 4th toe does not prevent some blood passing to the cushion, for stimulation of the lateral plantar will then cause pallor in it.

The evidence that the pallor is a passive result of the flushing will be given in § 2, but a curious result indicating this may be mentioned here. Sometimes when the nerve on the proximal artery side is stimulated there is first flushing spreading over the mid line and leaving only an "oval" pale area, then there is free secretion on approximately the half of the cushion of the toe, and as this occurs the pale area on the opposite side becomes larger. This suggests that the fluid removed from the deep capillaries in secretion reduces the blood-pressure in the dermal venous network and that this reduction affects the vessels of the opposite side because they are passively dilated, but is insufficient to affect those of the same side which are dilated by antidromic action.

The deep external plantar nerve supplies the plantar muscles. As I have said (Part I, p. 433), its connexions with the superficial plantar nerves, and so with the pad and toes, are variable, and it commonly sends them no fibres visible on dissection. I mentioned one case in which it sent an obvious strand to the 4th digital nerve. In this case it caused free secretion, sometimes accompanied by pallor and sometimes by flushing in the half of the toe supplied by the digital nerve. In another case it caused moderate secretion also accompanied either by pallor or flushing in the medial half of the 5th toe. In the eight experiments made, it always caused distinct, and sometimes great, pallor in the medial part

of the foot and less pallor, and sometimes a doubtful pallor, in the lateral part of the foot. Usually the inner side pad and the 2nd toe were most affected.

Apart from these changes, the areas affected varied in different experiments and with successive stimuli. Sometimes the 3rd toe was markedly paler and not infrequently the anterior part of the mid pad, and posterior part of the cushions of the 3rd and 4th toes became paler than the rest of these regions.

If the stimulation is prolonged for a minute or more, the paler parts may recover some tint, and after the end of the stimulation there is generally more or less flushing of the whole foot. When flushing is produced in a part of the foot by stimulating a superficial plantar nerve, the flushed part pales much more slowly than the rest on stimulating the deep external plantar. Thus with decreased capillary tone, a considerable decrease of local blood-pressure has relatively slight effect on capillary diameter. In most of the experiments the motor nerves were not paralysed and a primary immediate pallor was caused by the contraction; except, however, for the prompt pallor, the results were the same when the cat was decerebrated and curarised.

Some pallor may be caused in a toe after section of its digital nerves, so that part of the pallor is probably due to the contraction of the deep metatarsal arteries. A part seems also to be due to dilatation of muscle vessels, but so far I have only made preliminary experiments on the question of antidromic action on muscle.

A point which deserves further investigation is that in the three experiments in which the deep external plantar was stimulated after giving ergotoxine there was no reversal of the effect on the foot. The effect was to abolish or nearly abolish the action of the nerve. The nerve still appeared to cause slight paling, but this was not quite certain and when one or two of the toes were made to flush by stimulating a superficial plantar nerve, the deep external plantar did not cause these to become pale. In one experiment, 20 mgms. of ergotoxine were injected into the jugular vein, and the effect was little if at all greater than with 3–5 mgms. Five mgms. (probably less) are sufficient to reverse the action of the sympathetic on the foot (cp. p. 60) so that the whole of the effect of the deep external plantar was in the metatarsal region. The results suggest that either there is no reversal in small arterial trunks, or that the effect of the reversal is counteracted by dilatation in the vessels of the muscles.

The *anterior tibial nerve*, as the deep external plantar, causes pallor; its effect is greatest on the 2nd toe, somewhat less on the 3rd and still

less on the 4th and 5th. I have found it to cause secretion in the medial half of the 2nd toe, possibly with more experiments slight secretion would be found over a more extensive area.

The *dorsal nerve of the foot, i.e.* the superficial peroneal and such filaments of the internal saphenous nerve as may run to it, usually has less decided and more variable effects. When first stimulated it usually causes slight pallor followed by slight flush, or pallor in the tips of the toes and in patches elsewhere on the pad and toes. After several stimulations, and sometimes on the first stimulation, it causes partial flushing or general flushing of varying degree, similar effects can be seen in the dorsal skin of the digits. On stimulating it I have observed slight secretion in the 3rd, 4th and 5th toes, and in a small part of the mid pad. The part which secretes most is not constant and secretion may occur in some parts only of the toes as in one-half, or in the middle. Repeated stimulation is required to determine the maximum area of secretion.

The internal and external saphenous nerves I have only stimulated in two experiments. In neither was there any distinct effect on the plantar surface though the internal saphenous nerve appeared to cause trifling pallor in the inner toes.

The posterior tibial and peroneal nerves. Except in two experiments, the motor nerves were not paralysed, so that the contraction caused immediate pallor. The results were however the same, apart from immediately pallor and less after-flush in two experiments in which the animals were decerebrated and curarised.

The posterior tibial nerve when first stimulated causes usually marked pallor of the pad and toes lasting 30 to 60 seconds and free secretion. The pallor then lessens and gives way to great reddening. If the stimulus is discontinued at the end of 30 to 60 seconds, reddening sets in almost at once. The flushing gradually lessens but often lasts a quarter of an hour. When the stimulus is repeated a number of times, the primary pallor decreases, and after a variable number of stimuli there is no pallor but a slow flush, very distinct but usually less than the late flush produced by the early stimuli.

The peroneal nerve causes pallor followed by flushing in the dorsal skin of the digital region. On the plantar surface it causes a variable degree of pallor. In a considerable number of cases the effect was slight and confined to the tips of the toes and to patches on the rest of the toes and the pad. In these the nerve was not stimulated at the beginning of the experiment and it is probable that the maximal effect was not obtained. Secretion was never obtained on the whole secretory surface,

but not infrequently there was a trifling secretion in isolated patches on one or more of the toes and on the pad. There was variable degree of after-flushing.

2. *The cause of the pallor accompanying antidromic flushing.*

I have so far assumed that the pallor in one part of the foot which accompanies antidromic flushing in another part is a passive pallor. This was not my first impression, for the degree of the pallor sometimes occurring strongly suggested reflex arterial contraction. Experiment, however, showed that pallor occurred when no reflex action was possible.

The most striking instance of pallor accompanying flushing is that produced by stimulating the superficial lateral internal plantar nerve a little proximally of the pad. This, besides causing intense pallor of the side pads (the most constant effect), commonly causes great pallor of the 2nd toe and as great or nearly as great pallor of the 5th toe. There is no detectable nervous connection between the stimulated nerve and the 2nd and 5th toes, so that there is no anatomical basis for supposing that it sends vaso-constrictor fibres to these digits, and as both its secretory and its antidromic fibres have a local action it is most unlikely that its vaso-constrictor fibres have a wide distribution.

Krogh (4) has recently supported the hypothesis that the nerve fibres form terminal networks—separate networks being formed by vaso-constrictor and afferent nerve fibres—and that stimulation of a small region at the periphery may cause either vaso-constriction or vaso-dilation in adjoining regions. The chief argument for this appears to be that a crystal of nitrate of silver placed on the web between two toes of a frog was found by Krogh, Harrup and Rehberg (5) sometimes to cause dilatation in the web of the adjoining toes. A crystal would for some time form a nearly saturated solution and in view of the close network of vessels in the frog's foot, a more reasonable explanation is, I think, that the solution passed either by the network or by diffusion from the veins in sufficient concentration to affect directly the vessels. Some time ago (6) I gave reasons for considering that peripheral sympathetic nerve networks if they occur at all can only be of very limited extent[1]. If a network existed and stimulation of a part of it caused impulses to spread to other regions, impulses would also spread out, and almost certainly spread farther, on stimulatin[illegible] of [illegible] branches

[1] The overlapping of fibres of adjoining nerve roots [illegible] This is due to plexus formation either in the nerve trunks [illegible] branches as with the afferent fibres of the skin of the [illegible]

running to and helping to form the network. Thus the overlap of the areas affected by adjoining peripheral branches would be great. In fact the overlapping of the effect of adjoining nerve branches is very small. I mentioned this in connexion with the innervation of the iris, the blood-vessels of the trunk and the hairs of the cat. It is somewhat greater in the stomach and intestine but is not extensive. It is small in the skin of the frog. We have seen in this paper how little overlapping there is in the secretory and vaso-dilator effect of adjoining digital nerves. The results do not show that small areas of networks do not exist, but they show that they must be extremely limited, and a slight outspread of stimulus on local peripheral nerve stimulation almost certainly occurs from axon reflexes, since the nerves divide at the periphery. It is then most improbable that the pallor which occurs in one part of the foot when another part flushes is due to the local dilatation of the vessels stimulating a part of a vaso-constrictor nerve network and so producing pallor elsewhere. I made, however, some direct experiments. (1) The web between the 2nd and 3rd toes was cut for two-thirds of the distance[1] from the free edge, the sciatic and internal saphenous nerves cut, and the lateral internal plantar branch stimulated. The stimulation still caused pallor of the 2nd toe. (2) 1 p.c. novocaine was injected into the lateral side of the 2nd toe; this abolished all secretory and antidromic effect on this toe (cp. p. 62). Stimulation of the lateral internal plantar nerve still caused paling of the 2nd toe with flushing in the usual regions. The change of tint in this form of experiment is however not very great since novocaine causes pallor in the injected region. Experiments were also made with ergotoxine which will be referred to later.

Section of the posterior roots of the lower lumbar and sacral nerves does not prevent the pallor, so that it is not a reflex from the central nervous system.

The pallor of the 2nd toe on stimulating the lateral internal plantar nerve is not prevented by section of its dorsal digital nerves, all the superficial plantar, the deep external plantar, the internal saphenous and the sciatic nerves.

If the pallor were an axon reflex it should be produced by electrical stimulation of the central ends of the nerves of the flushing region. But I have not found, after section of the sciatic and internal saphenous nerves, any certain pallor in the 2nd and 5th toes on stimulating the central ends of the plantar or dorsal digital nerves of the 3rd toe, or the central end of the artery supplying it.

Lastly I have made some experiments on the effect of injecting ergo-

[1] Section further than this cut the main vein of the digit.

toxine. As is well known Dale(7) has shown that ergotoxine paralyses vaso-constrictor fibres in the cat; he mentions an experiment in which after 5 mgms. of "cornutine," stimulation of the lumbar sympathetic, which previously caused marked pallor, caused slight but distinct flushing.

The results were quite decisive. Ergotoxine increases both the flushing and the pallor caused by stimulating the superficial plantar nerves. The pallor was rather less regularly increased than the flushing. In one experiment (body weight 2·5 kgs.) I injected into the jugular vein 10 mgms. of ergotoxine phosphate in four successive doses in the course of three-quarters of an hour and then 10 mgms. in one dose. Throughout, the superficial lateral internal plantar nerve caused brilliant flushing in the usual areas, great pallor of the side pads, good pallor of the 2nd toe, rather less of the 5th toe. At the end of the experiment the effect was a little slower in developing than at its best, but was more marked than it had been before ergotoxine was given.

It is then, I think, clear that the pallor accompanying antidromic vaso-dilation is passive and due to the dilated vessels withdrawing blood from the adjoining regions.

Dale found that a larger dose of ergotoxine was required to paralyse the motor nerves to the base of the bladder, and the pilomotor nerves than to paralyse the vaso-constrictor nerves. For my purpose there was no object in giving a minimal dose, but I may mention that the first dose of 1·5 mgms. increased the effect of the internal lateral plantar both on flushing and secretion, and in another experiment the same effect was obtained with a first dose of 2·7 mgms. (body weight 1·3 kgs.). Five mgms. of ergotoxine (the smallest dose tried) reversed the action of the lumbar sympathetic on the foot. The pilomotor fibres were paralysed by 20 mgms., the motor nerves to the bladder were not quite paralysed by this dose. The larger amounts were not however given at one time.

Passive pallor alone does not, however, always account for the relative pallor in different parts of the foot. Some difference of capillary tone is required for this. Since differences undoubtedly occur it is not, I think, worth while discussing the particular cases.

The maximum pallor is much greater than any that has been described as accompanying hyperæmia elsewhere. This I attribute to the comparatively long course of the deep metatarsal and digital arteries and to their comparatively few arterial anastomoses, so that copious blood flow through one branch causes a great decrease of blood flow in adjoining branches. In the skin the arterial anastomoses are many. It need hardly be mentioned that the flushing of the pad and toes has little or no affect on the blood-pressure of the large arteries since it does not appreciably reduce the total resistance.

The disappearance of blood from the superficial vessels which causes the pallor might be produced in one of two ways, or by a combination of these, (*a*) the decrease of arterial blood-pressure might lead to a decrease of capillary diameter, or (*b*) the decrease of arterial diameter might prevent the corpuscles from passing through but not prevent plasma from passing, so that the corpuscles would be driven out of the capillaries and veins—a form of pallor which was said by Lister to occur sometimes in the frog's web.

On the whole the phenomena seem to me to require a shrinking of the capillaries as the blood-pressure is reduced for the following reasons:

(*a*) Complete pallor is commonly obtained by clamping the external iliac artery, and not infrequently by stimulating the lumbar sympathetic. It is possible that on clamping the iliac artery, the peripheral arteries contract sufficiently to stop the passage of corpuscles, but until this is shown its occurrence may be doubted. Further, if an artery contracts sufficiently to stop the passage of corpuscles, the corpuscles will rapidly block the artery, and it may be doubted whether in the intervening stage, plasma could pass in sufficient quantity to drive the corpuscles out of the capillaries and dermal venous network.

(*b*) The pallor progresses smoothly without any sudden change and, *as I have said, it is sometimes slow and may take a minute to reach its* maximum. With progressive decrease of arterial lumen, the stoppage of the corpuscles would be sudden and one would expect a more abrupt change than actually occurs.

(*c*) In the pallor accompanying antidromic flushing the small efferent vein of the cushion of the toe usually remains distinct. It hardly seems reasonable to suppose that the washing out would so often proceed to the extent of removing blood from the dermal venous network and yet leave corpuscles in the small efferent vein. The appearance suggests the continuance of blood flow though to a greatly restricted degree.

Whilst then in certain conditions it is inevitable that there should be some washing out of the corpuscles by a plasma stream, I think that the chief cause of pallor is the shrinking of the capillaries and dermal venous plexus in consequence of decrease of internal pressure.

In any case the experiments show that the volume of blood in the cat's foot is largely dependent on the local arterial blood-pressure. This is, of course, an old theory applied to a particular case, but it is not in agreement with the results obtained by Roy and Graham Brown (8) in the frog's web and other tissues, nor with those obtained by Krogh (9)

in the frog's web and tongue, and in striated muscles. According to these the diameter of capillaries and the volume of blood in them are practically independent of arterial blood-pressure in normal conditions.

Roy and Graham Brown found that the change in capillary diameter caused by increasing the external pressure was usually too slight to admit of measurement, but sometimes was 10–15 p.c. I found (10) at times definite though slight variations of diameter accompanying variations of arterial pressure. Krogh observed practically no change in the size of the capillaries of the web and tongue of the frog with varying arterial pressure, and he found (11) that normal arterial pressure was insufficient to open many of the capillaries of striated muscle. Krogh, however, noticed that capillaries are constantly varying in size, and that a dilated capillary yields readily to pressure so that there should be a variation in the diameter of some capillaries when the arterial pressure varies. The probability is that capillary tone varies greatly in different tissues. Each stage of tone may be regarded, not as a continued contraction but as being a different physical condition, such as has been shown to exist both in unstriated and striated muscle. Roy and Graham Brown argued that the elasticity of capillaries must vary in different degrees of contraction.

3. *Notes on the action of drugs.*

Novocaine has been found to paralyse afferent nerves before the motor nerves of skeletal muscle. It seemed then possible that the antidromic vaso-dilator fibres of the plantar nerves might be paralysed before the efferent sympathetic fibres. The injection of 0·1 to 0·2 c.c. of 0·5 to 1 p.c. novocaine under the plantar surface of a digit about the level of the 2nd phalanx rapidly prevents stimulation of the plantar nerves proximally of the pad from causing flushing of the cushion of the injected toe. The rest of the innervated area flushes. The sympathetic secretory and vaso-constrictor fibres are also paralysed. To observe the effect on vaso-constrictor fibres a plantar nerve branch which does not supply the pad should be taken. The duration of the paralysis depends naturally upon the amount of novocaine injected and its concentration. With the amount given above the paralysis lasts 10 to 30 minutes. In recovery I have not found any certain difference in the time at which flushing and secretion begins. If there is any differential action it can, I think, only be slight. A similar amount of novocaine injected into the mid pad prevents the posterior roots of the 7th lumbar nerve from causing flushing in the mid pad, but not in the side pads or toes. If, however, the injection is deep in the mid pad, the underlying plantar nerve branch is affected, and the adjoining halves (approximately) of the 3rd and 4th toes do not flush on stimulating the posterior roots. The injection causes local pallor during the paralysis, apparently by arterial constriction.

Nicotine. An intravenous injection of 20 mgms. of nicotine does not prevent the posterior roots from causing flushing. The flushing is however

decreased. The decrease may be attributed to lowered blood-pressure in consequence of paralysis of the peripheral ganglia.

Curari. As found by most observers an amount of curari sufficient to paralyse the motor nerves of skeletal muscle has little or no effect on antidromic flushing. If sufficient is given to paralyse the peripheral ganglia and so cause a great fall of blood-pressure, the antidromic effeot is much reduced.

Atropine. Ostroumoff found that atropine did not prevent the sciatic nerve from causing a rise of temperature in curarised dogs. As the rise of temperature is mainly due to antidromic action, the result is fair evidence that neither the atropine nor the curari given prevented antidromic action. Reid Hunt in plethysmograph experiments found that atropine did not prevent antidromic increase of volume of the hind limb. And intravenous injection of 10 mgms. atropine sulphate does not prevent the lower lumbar posterior roots from causing flushing in the cat's foot. In two experiments there appeared to be a slight decrease in effect, but as the blood-pressure was not taken, the decrease may have been due to fall of blood-pressuro. Atropine, however, does affect the result of stimulating the superficial plantar nerves, favouring the occurrence of primary pallor, a result I deal with in another paper.

Acetyl-choline. Dale and Richards (3, p. 135) showed that intravenous injection of ·001 mgm. of acetyl-choline caused for a short time flushing of the pads of the cat's foot. I made one experiment, injecting into the mid pad 0·1 c.c. of a 0·1 p.c. alcoholic solution. The injection caused free secretion and considerable flushing in the mid pad. Stimulation of the 7th L.-posterior roots increased the flushing slightly but distinctly. The action of ergotoxine I have mentioned earlier (p. 60).

These results suggest that only drugs which abolish nerve excitability prevent antidromic vaso-dilation; the effect of other drugs depending on their effect on blood-pressure.

4. *Theories of antidromic action.*

The conclusions arrived at in Part I were that antidromic vaso-dilation is produced either by a special kind of afferent fibre ending in the capillaries or by metabolites set free in cells by impulses passing down afferent nerve fibres. These conclusions are, I think, strengthened by the results given in this paper, and they tend in addition to show that the capillaries affected in the foot are not confined to those of the skin but include those of the subcutaneous tissue and probably also the dermal venous network. The theory of action by metabolites is that

which appeals to me most. I have made some experiments on the local injection of blood from flushed areas and of skin extracts, but so far with varying results. Whilst the final decision must rest on further experiments, it will clear the ground to consider briefly the theories which have been put forward and the facts on which they are based.

The history begins with the early observation of Schiff that section of the nerve roots supplying a limb caused a rise of temperature in the limb. The method was that introduced by Cl. Bernard to show the presence of vaso-constrictor fibres, and Schiff argued that there were direct vaso-constrictor fibres in the anterior roots of all the spinal nerves. The facts were disputed and the theory of direct fibres gradually discredited. The discovery by Goltz (1874) that crimping the sciatic nerve caused a rise of temperature in the hind limb was the origin of numerous observations on the question of the existence of vaso-dilator fibres in the sympathetic, and of direct vaso-dilator fibres in the spinal nerves. As is well known a new turn was given to the question by Stricker (1876) who found that stimulation, chiefly mechanical, of the posterior roots of the nerves of the hind limb caused a rise of temperature in it. The main result was confirmed by Bonuzzi, Bornezzi, Morat and Werziloff, and all attributed it to the presence of vaso-dilator fibres differing only from the known vaso-dilator fibres in that they left the spinal cord in the posterior roots and ran direct in the spinal nerves. Morat(12), besides observing that visible flushing in the foot could be obtained by ordinary electrical stimulation of posterior roots, stated that flushing was obtained 8–10 and 15 days after their section. In the fuller account published a little later(13) he only mentioned dilatation on stimulating the posterior roots as being obtained after degeneration
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earlier statement was based. He considered, how... in recovery I have not
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in the peripheral ends of cut posterior roots had been confirmed by several recent observers. This absence of degeneration I had also found, and in consequence in giving an account of the sympathetic system I said (15) that the effect could not be caused by fibres arising from the spinal cord, and I was inclined to believe that the effects described were not due to fibres in the spinal nerve roots. Later the way to reconcile the results occurred to me, viz. by impulses passing backwards along the afferent fibres after the manner of that in the axon reflexes which I had found in sympathetic fibres. The protracted rise of temperature which had been described in the muscles led me to believe that the effect was due to metabolites set free in muscle spindles, the metabolites causing vascular dilatation. Bayliss (16), using the plethysmograph method, showed that both electrical and mechanical stimulation of the posterior roots caused marked increase of volume of the limb of the dog and he attributed the vaso-dilator action, as previous observers had done, to efferent nerve fibres. I communicated my views to him through the Head of the Laboratory (Prof. Starling) and suggested stimulation and histological examination of the posterior roots after they had been cut and allowed to degenerate. Bayliss's subsequent experiments (17) confirmed the theory of vaso-dilation by afferent fibres and he showed that various other conceivable theories were not tenable. He found, however, that stimulation of the posterior roots caused a considerable dilatation of the foot and a very small dilatation in the leg after the skin had been removed and the foot cut off. In consequence he considered that the effect was almost entirely in the skin and that it was probably due to the endings of the afferent fibres in the arteries acting both as motor and sensory organs. The question of the degree of dilatation in muscle can hardly be considered as settled, for only one experiment was made, and removal of the skin would probably interfere with the circulation in the muscle. In the foot there is muscle, fat and gland tissue as well as skin and as mentioned above there is evidence that the dilatation is not confined to the skin.

N. Bruce (18), following up some results obtained by Spiess, observed that oil of mustard locally applied ceased to cause dilatation of the conjunctival vessels after degeneration of the conjunctival nerves. He concluded that the normal dilatation caused by oil of mustard was due to stimulation in the epithelium of the endings of nerve fibres which divided and sent one branch to an artery, *i.e.* the dilatation was held to be produced by a peripheral axon reflex. There are several possibilities as to the action of oil of mustard. It might affect the deeper tissues as

well as the epithelium, and set free metabolites partly by direct action and partly by stimulating sensory nerves. The less action after nerve degeneration might be due to the absence of nerves to stimulate or to a decrease in the responsiveness of the tissue.

Bardy (19) confirmed in the main Bruce's results, though he did not find a complete absence of hyperæmia on applying oil of mustard after degeneration of the sensory nerves. He modified Bruce's theory by interpolating a sympathetic nerve cell in the centrifugal part of the axon reflex. This theory I consider untenable. It is based on the action of nicotine. Bardy found that repeated local instillation of 2 p.c. nicotine, after partial local anæsthesia, greatly reduced the effect of the oil of mustard and paralysed the vaso-constrictor effect of the cervical sympathetic on the nictitating membrane. The repeated application of 2 p.c. nicotine would annul the conductivity of the sensory fibres, and the condition would be similar to that produced by cocaine. The paralysis of the cervical sympathetic would be caused either by the abolition of the conductivity of the vaso-constrictor fibres by nicotine directly applied, or to the absorbed nicotine paralysing the nerve cells of the superior cervical ganglion. The other result on which Bardy relied was that intravenous injection of 60 mgms. of nicotine abolished the hyperæmic effect of oil of mustard. This is the natural result of the great reduction of blood-pressure caused by a large dose of nicotine. In fact, Bardy notes that there was a similar reduction in the effect of oil of mustard on ligaturing the common carotid, a procedure which causes less local fall of blood-pressure than that caused by the injected nicotine.

Gaskell (20), who took Bayliss's experiments to show that antidromic action was solely in the skin, suggested that the sensory nerves might cause the formation of acid metabolites in the epidermis and that the vaso-dilation was due to the action of these. Ebbeke (21) investigated the effect of mechanical stimulation on the blood vessels—chiefly in the skin. The red stripe which is produced by rather strong stroking he considered was a capillary dilatation produced by metabolites of the epidermic cells, and he suggested that antidromic action might also be of the same nature.

The effect of the posterior roots on the vessels of the frog's web is still obscure, for not all observers find positive effects, and there are differences in the accounts of those who do find them. But according to Doi (22) the arteries and capillaries of the web, and according to Krogh, Harrup and Rehberg, the arteries and some of the capillaries dilate on stimulating the posterior roots. The observations are perhaps somewhat in

favour of a direct action on the arteries, but it is obvious that as the arteries are practically in the same plane as the capillaries, metabolites might affect both.

Krogh, Harrup and Rehberg from the persistence of local reactions in arteries and capillaries in the web of the frog after excision of ganglia and section of the sciatic adopt tentatively the view that a certain number of nerve fibres are kept alive after nerve section by peripheral nerve cells. In view of the histological evidence of degeneration obtained by Euglein in the ear of the rabbit and by myself in the sartorius muscle of the frog and in view of the absence of recognisable nerve cells in the periphery, it is I think more probable that the persistent reactions they found were either due to conduction from cell to cell in the vessels or to aberrant nerve cells. I once found a small group of nerve cells of the type of sympathetic cells in the upper part of a frog's sciatic nerve.

Finally I may say a word or two on the mode of reaction of the capillaries. The theory that their contraction and expansion is due to a variation of tonic contraction of branched cells (Rouget's cells) surrounding them has not, I think, at present any satisfactory basis. In the cat's foot, and in such other mammalian tissues as I have examined, the cells in connexion with the capillaries seem to me indistinguishable from the immediately adjoining connective tissue cells, and it is rare to find one which has the appearance of surrounding the capillary with its processes. It is not claimed that Rouget cells form more than a most imperfect coat to the capillary. If then, the tone resided in these cells, there would be in the more or less tonic normal state, a series of bulgings of the distensible epithelioid coat whenever the internal pressure was greater than the external. The main arguments for the theory are the improbability of the epithelioid wall having contractile power, and that in a few cases in the frog the capillaries have been found to contract earlier and more strongly in the region of an external cell. On the other hand it may be said that there is no improbability in the thin epithelioid wall shrinking or expanding with variations of surface tension such as can be caused by minute amounts of chemical bodies, nor in each change of surface tension involving a different state of distensibility and as regards the localised contraction Roy and Graham Brown (8) definitely state that the capillary tube may expand and contract as a whole.

Summary.

The effects on the colour of the pad and toes of the foot of the cat produced by stimulating the several nerves are given. The chief results as regards antidromic action are:

The superficial plantar nerves, notwithstanding the presence in them of sympathetic vaso-constrictor fibres, nearly always cause marked

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flushing in the pad and toes on strong or repeated stimulation. Each plantar digital nerve causes secretion and flushing more or less confined to its own half of the cushion of the toe.

Stimulation of the digital nerve on the side of the toe having the proximal part of the artery does not cause flushing on the whole of the opposite side of the toe although this is supplied with blood from a distal branch of the artery. This confirms the view that antidromic impulses do not cause dilatation of small arterial trunks.

The secretion obtained by stimulating a plantar digital nerve never spreads more than about a millimetre into the opposite side of the cushion of the toe.

The flushing may be similarly restricted. The degree to which it spreads to the opposite side depends on two factors. The most important is the degree of tone in the vessels of the opposite side. If this has been reduced by antidromic action, the whole of the opposite side of the toe may flush.

The other factor is the position of the afferent vein of the dermal venous plexus; this is placed on the side of the toe which has the distal artery, thus the dilatation spreads farther from proximal to distal artery side than in the reverse direction.

On stimulating one of the superficial plantar nerves, all parts of the pad and toes which do not flush become paler. The pallor is not due to outspread of stimuli by a nerve network or to an axon reflex—amongst other reasons because, both flushing and pallor are more marked after paralysis of the vaso-constrictor fibres by ergotoxine; thus local arterial pressure in the foot has a great effect on capillary diameter.

The deep external plantar nerve ordinarily causes pallor of the foot by an action in the metatarsal region; this is not reversed by 20 mgms. of ergotoxine phosphate.

The successive stages of the theories of the mode of production of vaso-dilation by the lumbar nerves are reviewed and the evidence which the facts afford of the mode of production is discussed.

CONCLUSIONS.

The flushing caused by antidromic nerve impulses in the pad and toes of the cat is due to dilatation in the capillaries of the skin and in all probability to dilatation of the dermal venous network and the capillaries of the subcutaneous tissue. The three together may be spoken of as the capillary system.

Antidromic impulses do not cause dilatation of the arteries from the

aorta up to the final arterial branches and probably not directly in the final arterial branches.

Decrease of blood-pressure combined with decrease of blood supply causes this capillary system to become nearly empty of blood and the size of the vessels to decrease. The degree to which this effect is produced with a given lowering of blood supply depends on the tone of the capillary system. If the tone has been reduced by previous antidromic impulses, great decrease of local blood-pressure causes slight change only in the capillary system. This is in harmony with the observations of Krogh that in the frog a low blood-pressure is sufficient to dilate a capillary the tone of which has been reduced by mechanical stimulation.

The facts at present known are insufficient to decide whether the antidromic vaso-dilation is produced by afferent fibres ending in the capillaries or by afferent fibres setting free metabolites. The indirect evidence seems to me to be in favour of the latter theory. On the metabolic theory the varying tone of the capillaries may be regarded as produced by physical changes in the epithelioid wall leading to a change in diameter and in extensibility.

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THE VASCULAR DILATATION CAUSED BY THE SYMPATHETIC AND THE COURSE OF VASO-MOTOR NERVES. By J. N. LANGLEY.

(*From the Physiological Laboratory, Cambridge.*)

The vascular dilatation caused by the sympathetic.

In early experiments (1) I found that stimulation of those spinal nerves which caused secretion in the fore or hind feet of the cat commonly caused also flushing of the skin of the foot, usually, but not always, preceded by pallor. Both the preganglionic fibres causing pallor and those causing flushing were found to end in the same sympathetic ganglia. At the time I regarded the results as evidence of the existence of vaso-dilator fibres in sympathetic nerves. In some subsequent experiments (2) I only found flushing after primary pallor. In the course of recent experiments on antidromic action I had occasion to stimulate the lumbar sympathetic and again obtained at times primary flushing. I noticed also that stimulation of the peripheral branches of the plantar nerves had a greater tendency to cause pallor after atropine had been injected. In consequence I have made some further observations on the effects of stimulation of the lumbar sympathetic on the colour of the foot.

The cats were anæsthetised first with chloroform and after tracheotomy with C.E. mixture. They lay unbound on the table and care was taken to avoid any pressure on the veins. The sympathetic was cut and stimulated below the 5th lumbar ganglion, the white ramus to the 6th ganglion (if present) was cut, or this ganglion isolated. When the sympathetic on both sides was prepared, the intestines were sometimes removed. Erection of the tail hairs, and contraction of the trigonum of the bladder served as accessory indications of nerve excitability.

The effects of stimulating the peripheral end of the lumbar sympathetic vary greatly in different cats. The predominant effect, as is known, is primary pallor. This is obtained in every experiment. The pallor may be complete and even the efferent vein of the cushion of the toe may disappear from view, or the pallor may be slight and unequal in different parts of the foot. Frequently there is some return of colour when the stimulus is kept on for 45 to 60 secs.; this may pass into increase of colour, or the increase may only occur when the stimulus has ceased. But occasionally some of the stimuli, even early in an experiment, cause

flushing in 5 to 10 secs. with no perceptible preceding pallor, and later becomes very marked. All variations occur between early flushing and that which only occurs after the stimulation has ceased. Occasionally also the pallor continues for a minute or more after the stimulation, and then only slowly gives way to a slight flush.

The flushing was generally slight in the pad, greater in the 3rd and 4th toes than in the 2nd, greater in this than in the 5th. The pad—especially the anterior part of the mid pad—sometimes becomes pale, whilst the toes flushed, and sometimes a portion of each toe became paler while the remaining part flushed. Thus in one case there was a distinct white patch on the proximal part of the cushion of each toe, on the side of the proximal part of the artery whilst the rest of the toe flushed. A weak stimulus appears to favour the production of pallor. In two experiments in which there was deep urethane anæsthesia, the feet were red and relatively slightly influenced by the sympathetic.

The varying results show that the change of colour is influenced by several factors. The early flushing might be due (*a*) to earlier or greater vascular contraction in another region, (*b*) to formation of vaso-dilator metabolites by sympathetic secretory fibres. These factors, it may be noticed, may also be operative in the increase of volume of visceral organs which have occasionally been observed on stimulating thoracic nerve roots. The late flushing might be due, as suggested by Roy and Graham Brown (3), to the previous constriction causing anæmia and so setting free vaso-dilator metabolites.

As I have said, atropine usually favours the production of pallor by the superficial plantar nerves, and it is an obvious explanation that it does so by paralysing the secretory nerves and thus preventing the formation of vaso-dilator metabolites. In order to test this, the lumbar sympathetic was stimulated before and after giving atropine (10 mgms. of the sulphate). I did not find that the vaso-constrictor action was increased by atropine, if there was any change it was somewhat lessened, but the late-flush and the after-flush were much decreased, and in the few cases in which a primary flush was obtained it was abolished by atropine. The primary flush was not however obtained sufficiently often to give any certainty that it might not sometimes occur after atropine. Further, before atropine was given, the flushing sometimes occurred without visible secretion or before visible secretion. But visible secretion depends greatly on the state of the epidermis. The sweat glands of a foot which secrete very feebly appear normal in microscopic sections, and it might therefore be argued that the sympathetic had not lost its action upon them.

The results, I think, show that the sweat glands in activity produce a vaso-dilator substance (or vaso-dilator substances) and that whether this causes increased blood volume or not when the sympathetic is

stimulated depends on the degree of simultaneous vaso-constriction. Further, since the main secretory part of the sweat glands is in the subcutaneous tissue, we may conclude that the chief dilatation is in the vessels of the subcutaneous tissue.

The production of vaso-dilator substances by the sweat glands is merely another instance of a widespread if not universal result of cell activity. Notable instances are those of the salivary glands and of muscle described by Barcroft and Kato (4). In the case we are considering, its occurrence weakens the evidence of the existence of sympathetic vaso-dilator fibres in the nerves of the foot. The evidence for such fibres rests mainly on the effect of ergotoxine found by Dale (5). Dale showed that ergotoxine paralyses vaso-constrictor fibres and he found that after injecting it (5 mgms. of cornutine) stimulation of the lumbar sympathetic, instead of causing pallor of the cat's foot, caused slight but distinct flushing. But he found that secretion was still abundant. In view of the effect of atropine given above, the flushing might then be due to metabolites set free in secretion. On testing this possibility, it was, however, negatived. Atropine given after ergotoxine did not prevent the sympathetic from causing flushing. One experiment was as follows:

Cat, anæsthetised with C.E. and urethane. Feet red. The lumbar sympathetic caused distinct but patchy primary pallor with fairly free secretion followed by flushing in the toes. After 10 mgms. of atropine sulphate, the sympathetic caused pallor much as before, but no obvious flushing. 5 mgms. of ergotoxine phosphate were injected, and a little later 10 mgms. of atropine sulphate. The sympathetic then caused a good flush. Adrenaline still caused a temporary fall of blood-pressure.

Supposed vaso-constrictor fibres accompanying the arteries.

It has been found in clinical practice that section of the outer sheath of the femoral or the brachial artery (peri-arterial section) abolishes in certain cases (? temporarily only) peripheral vaso-constriction such as occurs in Raynaud's disease. From this it has been concluded that sympathetic vaso-constrictor fibres pass to the periphery by the arteries. In former papers I have stated that sympathetic fibres run to the trunk and limbs by the spinal nerves and not by the arteries. In view of the clinical result just mentioned I have made some further experiments. The lumbar sympathetic was prepared as described above, and stimulated before and after section of the crural and sciatic nerves and the colour of the foot observed. The pallor of the foot obtained before section was abolished by the section. As a further test, ergotoxine was injected, and the lumbar sympathetic stimulated. On the side with cut nerves it had no effect, on the side with intact nerves it caused marked flushing.

Two experiments were made on the effect of successive section of the

peripheral nerves. After section of the posterior tibial nerve at the tendo Achillis the sympathetic still caused complete pallor of the pad and toes, though not with every stimulus. Section, in addition, of the musculo-cutaneous (n. peron. superf.) on the dorsal surface of the foot greatly reduced the pallor obtained, and it was chiefly in the 2nd toe. Additional section of the anterior tibial nerves (n. peron. prof.) just above the ankle nearly abolished the effect of the sympathetic. Finally, after section of the anterior tibial nerve where it separates from the musculo-cutaneous, the sympathetic perhaps caused a trifling pallor, but it was too slight to be certain about.

The plan of sympathetic innervation of the arteries, I take to be, that the ganglia of the sympathetic chain send fibres to the immediately adjoining arteries (aorta and vertebral arteries), and that the peripheral arteries receive small filaments at intervals from the nerves accompanying them, each filament supplying a portion only of the artery. So far then as peri-arterial section abolishes peripheral vaso-constriction, its effect must, I think, be due to some other cause than section of nerve fibres running to the periphery in the arterial sheath. Possibly it may be due to section of afferent fibres, or to the contracted part of the artery being near the point of peri-arterial section.

Summary and Conclusions.

Stimulation of the lumbar sympathetic commonly causes flushing of the cat's foot after primary pallor, and occasionally flushing with no appreciable preceding pallor.

Atropine decreases the degree of flushing and prevents, or nearly prevents, the occurrence of primary flushing. Thus the flushing is largely due to metabolites set free by the sweat glands in secretion. Atropine does not, however, prevent ergotoxine from converting the vaso-constrictor action of the sympathetic into a vaso-dilator action.

After section of the sciatic and crural nerves, stimulation of the lumbar sympathetic causes no appreciable pallor of the foot, and after ergotoxine has been given it causes no flushing. Thus peri-arterial section in man, so far as it relieves peripheral vaso-constriction, in all probability does not do so by severing nerve fibres running with the arteries to the periphery.

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ON THE OVARIAN FACTOR CONCERNED IN THE OCCURRENCE OF ŒSTRUS. By F. H. A. MARSHALL, F.R.S., and W. A. WOOD, F.R.C.V.S.

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In a paper by Marshall and Runciman the view was tentatively expressed that the occurrence of the procestrum and œstrus in dogs does not depend upon the presence of ripe Graafian follicles in the ovaries. This conclusion was suggested as a result of three experiments in which all the visible follicles in the ovaries were pricked or cut by a needle or knife a short time (3 to 8 weeks) before a "heat" period was due. Each of the three bitches experienced normal heat at or very shortly after the expected time. The animals were killed from one to three weeks later, when the ruptured follicles were found to have become converted into structures identical with or resembling corpora lutea but (at least in one case) with abnormally large cavities.

In criticism of the suggestion that these experiments indicated that the phenomena of "heat" are due to ovarian organs other than the epithelial cells of the ripe follicles Robinson has remarked with some justice that the cells of the ruptured follicles were not necessarily functionally interfered with, in spite of the fact that they subsequently became converted into luteal cells; moreover, the experiments did not show at what precise period the hypertrophy and transformation occurred. Robinson states further that in ferrets heat is only experienced when ovarian follicles are of a certain degree of development which he calls the "pre-inseminal" stage. At this time the follicles "appear to take on the function of providing the secretion which is responsible for the phenomena of procestrum and œstrus." Lastly in the ferret follicles of the pre-inseminal stage may remain present for a long period without undergoing atrophy, and when this is so œstrus persists for a corresponding period which may be very extended (cf. Marshall, 1904).

The experiments described in this paper were conducted on the same lines as those previously described but with some modification in method in two cases.

1. A Pomeranian bitch was seen to be on "heat" from March 4th to 10th, 1921. Since the normal œstrous cycle in the dog is six months

another heat period was due in September. The bitch was operated upon on August 5th, the ovaries being exposed and the visible follicles cut with a knife. She began to show procestrous bleeding on September 9th and then went through the normal procestrous and œstrous changes (so far as could be seen externally), the flow of blood from the vulva finally ceasing on September 18th. She was killed on October 6th. Thus the heat period occurred at the expected time and the experiment was in every respect like the earlier ones of Marshall and Runciman. Histological examination of the ovaries and uterus showed the typical indications of mid-pseudopregnancy. The ovaries contained numerous corpora lutea which were well vascularised but the luteal cells were partially vacuolated (where the fat had been dissolved out by alcohol) and were evidently just entering on regression. The uterus showed great glandular development but the epithelium of the glands was on the way to becoming cubical. The condition recalled the 31 day stage of pseudopregnancy described by Marshall and Halnan. It is clear that the experiment is open to the same criticism as the earlier ones of Marshall and Runciman since it is possible that the follicle cells were not destroyed and quite conceivably continued to function after being cut into.

2. An Aberdeen terrier bitch was seen to be "on heat" from March 4th to 15th, 1921. External bleeding at the vulva decreased on the 7th but there was a trace of blood as late as the 15th after which bleeding finally ceased. The bitch was operated upon on August 5th, 1921, but instead of the ovaries being cut with a knife the visible follicles were cauterised by a red-hot needle. The bitch was kept under careful observation for sixteen months during which time no heat period occurred. Subsequently the bitch was examined less regularly but it is unlikely that heat could have taken place without being observed. Signs of heat were however noticed early in May, 1923. The bitch was eventually killed on June 7th when it was found to be in an advanced stage of pseudopregnancy, the uterus—more especially one horn—containing a large quantity of glandular secretion probably comparable in nature to the "uterine milk" of Ungulates, while the mammary glands had also undergone much growth and were actively secreting milk which was freely discharged on the glands being cut. Sections through the ovaries showed numerous corpora lutea with much vacuolated luteal cells as well as many developing follicles. The two horns of the uterus differed considerably in histological appearance. One was considerably more distended than the other, and transverse sections of the distended horn showed a great development of the uterine glands which had however begun to undergo pseudopregnant retrogres-

sion. The wall of the cavity was much folded. The whole appearance was not unlike that of a rabbit's uterus rather more than mid-way through pseudopregnancy. The cavities of the glands were often very large and often contained a secretion and sometimes also what appeared to be desquamated epithelial cells. The epithelial lining was cubical rather than columnar, but in some of the deeper parts of the mucosa appeared to have undergone almost complete destruction. The same was true of the other or less-distended uterine horn. Here the glands were for the most part smaller, and as a general rule resembled the deeper glands of the distended horn. Enlarged capillaries containing red corpuscles were numerous in parts of the stroma of both uterine horns. The sections were in a general way similar to those of the 38 and 43 day stages of pseudopregnancy described by Marshall and Halnan. The mammary glands also presented a condition of very pronounced pseudopregnancy and both the secretory alveoli and the ducts contained an abundance of milk.

3. A fox terrier bitch was kept under observations from March, 1921, onwards. She experienced normal procestrum (with hæmorrhage at the vulva) in May, in September, and in March, 1922. The œstrous cycle was thus a little irregular, but it is not unusual for dogs, especially those of the smaller breeds, to experience a somewhat shorter cycle in summer than in winter. The bitch was operated upon, in precisely the same way as in case 2, on June 13th, 1922, or three months after the last heat period and therefore from one to three months before a new period was due in this animal. All the follicles seen on the surface of the ovaries were cauterised with a red-hot needle. The bitch was kept under close observation and did not come "on heat" again until March 2nd, 1923, when procestrous bleeding commenced. Procestrum was followed by œstrus and the bitch was served by a dog on March 9th. She became pregnant and gave birth to three pups on May 14th after a pregnancy very slightly longer than the normal. She was killed on June 7th. Sections through the ovaries showed corpora lutea with much vacuolated luteal cells obviously in a state of advanced retrogression. The mammary glands were still active and contained much milk. The uterus had not yet undergone involution but contained a quantity of decidual tissue. The unusually long time taken over involution was probably correlated with the fact that the pups died soon after birth and consequently normal lactation did not occur, it being known that suckling favours rapid involution; this has been demonstrated more especially in the case of the guinea-pig by Loeb.

The experiments, few in number as they are, show clearly that

destruction of the larger follicles by cauterisation led to a different result from pricking the follicles or cutting them with a knife. They support Robinson's contention that the phenomena of procestrum and œstrus only appear in the presence of follicles which have attained a certain stage of development, which he calls "pre-inseminal maturity," and that the phenomena are due to some secretion produced by the follicles at that particular phase. "In all young animals group after group of follicles grows and dies but no signs of heat appear because none of the groups have attained to the phase of growth during which they form the heat-producing secretion. Again, in sexually mature and adult guinea-pigs and ferrets group after group of follicles grows and dies without attaining the stage at which the heat-producing secretion is formed; but once a group of follicles has attained to that stage which is shown in the ferret when the cumulus epithelium begins to show signs of separation into an inner and outer group of cells, the phenomena of the procestrum followed by those of the œstrus appear." It was probably shortly before this stage was reached that the bitches' follicles were cauterised in the experiments described above. The prolonged postponement of heat could not have been a mere "post-operative effect," since the animals very rapidly recovered from the operation and lived perfectly healthily until their death. Beyond the existence of a small amount of scar tissue no trace was found of the destroyed follicles in the ovaries of the bitches after slaughter.

With regard to the possibility that "heat" is due to an internal secretion from the ovarian interstitial cells the evidence is mostly negative. We have not been able to identify such cells with any certainty in the bitch. Aimé writing in 1907 says they have not been seen in the ovaries of the adult pig, sheep, dog or man. Aeschner also states that no interstitial cells are present at the time of the first heat in the dog but only before puberty. Robinson says that in some bitch ovaries in his possession interstitial cells are undoubtedly present in the form of strands of cells and scattered cells but that they do not form so prominent a feature of the ovarian structure as in the cat and ferret which, unlike the bitch, do not ovulate spontaneously during œstrus. Robinson however says nothing about the age of the animals nor about the stage in the cycle at which the cells were seen.

It is well known that the uterus after ovariotomy undergoes atrophy; in the rat this condition is very marked at the end of six months (Marshall and Jolly). It seems unlikely that the organ having once become definitely atrophic could be restored to the normal condition,

and the inference is probably correct that in the last two experiments described above the normal uterine nutrition was maintained in spite of the temporary inhibition of the œstrous cycle, that is to say, the ovarian secretion which is responsible for maintaining the uterus and preventing it from lapsing into the atrophic condition continued to be produced and to exert its usual influence over the uterus just as it does during the normal anœstrum.

It may probably be concluded therefore that the ovaries of the adult animal produce at least three kinds of internal secretions which differ from one another both quantitatively and qualitatively:

(1) The secretion which is responsible for maintaining the normal nutrition of the uterus throughout the entire cycle and preventing the organ from lapsing; this secretion is possibly produced by the smaller follicles or by the interstitial cells or by both combined; (2) the heat-producing secretion which is associated with the pre-inseminal phase of follicular maturation; (3) the secretion produced by the corpus luteum which is responsible for uterine and mammary hypertrophy during pregnancy and pseudopregnancy.

In further support of these conclusions certain other observations may be cited. Pugh in describing the condition of nymphomania in cows and heifers, an abnormal state in which the animals appear to remain continuously "on heat," says that the larger Graafian follicles have become cystic, and that this condition appears to stimulate the growth of the epithelial cells and so possibly favours the production of the internal secretion which is responsible for the continuous œstrus. Lothe also has found that when heat in cows is continuous or too frequent it is associated with cystic ovaries.

Various investigators such as Steinach and Holzknecht have found that irradiation of the ovaries by the X-rays causes the degeneration of the follicles or at any rate of the larger ones, the extent of the degeneration appearing to depend upon the dosage. (Steinach and Holzknecht however state that the interstitial cells do not degenerate but may even hypertrophy.) Werner found that irradiation in women caused amenorrhœa but that menstruation almost always reappeared subsequently and some of the women afterwards gave birth to normal children. It is clear therefore that the smaller or less mature follicles were not destroyed. This result is exactly comparable to what we believe to have occurred in our last two experiments on dogs. On the other hand, McIlroy found that the normal nutrition of the uterus of the rabbit could be preserved by ovarian grafts in which the follicle cells had de-

generated, and of the possible secretory elements, only the interstitial cells remained There is no evidence however that the animals came "on heat"

That "heat" is not due to the corpus luteum, which acts rather as an inhibitor of that condition, is now generally admitted (see Marshall, 1922) This fact was recognised long ago by Zschokke who initiated the practice now adopted in many different countries, of squeezing out the persistent corpora lutea of cattle in order to favour their coming "on heat" Moreover, at the commencement of heat corpora lutea (or at any rate newly formed ones) are not present in the ovaries It may be taken as established however that the œstrous cycle comes to an end at once and the uterus atrophies after a few months if the ovaries be removed

Summary

The experiments upon dogs show that if all the Graafian follicles visible on the surface of the ovary are cauterised, and therefore probably destroyed, a short time before a heat period is due, then the period is missed and there is a prolonged period of anœstrum which may extend over the duration of two or more normal œstrous cycles That the uterus does not undergo atrophy is shown by the subsequent reappearance of heat and the resumption of normality If the follicles are cut into without being destroyed, heat is not postponed or only slightly so

The general conclusions reached are that procœstrum and œstrus are due to an internal secretion of the ovary produced by the follicles during the pre inseminal stage of development The secretion which is responsible for uterine and mammary hypertrophy during pregnancy and pseudopregnancy is different, and is produced by the corpus luteum There is also an ovarian internal secretion which is responsible for main taining the normal nutrition of the uterus and preventing it from lapsing into an infantile or atrophic condition, and this is produced by the smaller follicles or by the interstitial cells The three above mentioned ovarian secretions probably differ from one another qualitatively as well as quantitatively

We are indebted to our laboratory assistant, Mr Tadman, for helping us with the above described experiments

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THE REGULATION OF RESPIRATION. Part I.

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In two previous articles(1), four respiratory centres were located, namely, the gasping centre at the nœud vital, Fig. 1 (5–6), the expiratory centre just above this, Fig. 1 (4–5), the apneustic centre which gives rise to inspiratory tonus (apneusis) and is placed at the level of the striæ acousticæ, Fig. 1 (3–4), and the pneumotaxic centre in the upper half of the pons, Fig. 1 (1–2), which produces respiration of normal type by periodically inhibiting apneusis. The present paper deals with the chemical, and afferent nervous, influences which regulate the activity of centres 1, 2 and 3.

Gasping. The influences affecting gasping may best be studied after all the higher centres have died, or have been eliminated by section of the brain stem at level 5, Fig. 1.

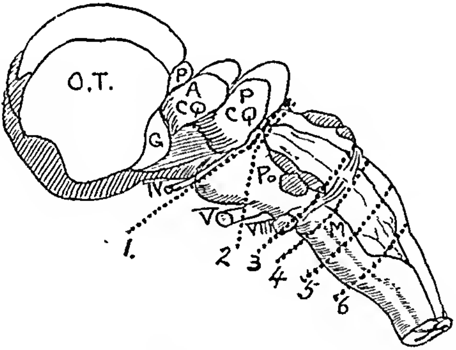

Fig. 1. Diagram of brain stem showing level of crucial sections.

When investigating the chemical influences, it is best to employ continuous ventilation of the lungs with various gases, as indicated in my second article(1). Even so, if the brain stem has been divided at

level 5. the unsatisfactory circulation resulting from this section complicates matters: while if such a section has not been made, it is difficult to be certain that the higher centres are completely out of action. This is a point of essential importance, since vagal stimulation, for instance, produces entirely different results on gasping, according as the higher centres are dead, or are simply in abeyance, so that they still retain some inhibitory power over the gasping centre. A good deal can, however, be learned from the study of gasping as it occurs during apneustic respiration, since the gasping centre is then well nourished, and the normal inhibitory power of the higher centres can be much diminished by vagotomy.

Although even after Section 5, gasping may continue for 15 to 30 minutes, well controlled results are difficult to obtain. The blood-pressure, already low, is steadily falling, and the circulation through the nœud vital is becoming more and more unsatisfactory. There is thus a constant tendency for O_2 to be so seriously lacking, and CO_2 to be so much in excess, that only when gasping is proceeding very briskly can we expect any increase of it by further diminishing O_2, or increasing CO_2. More often, one is compelled to seek evidence by improving, rather than by aggravating the condition of the blood. Further, the activity of gasping often varies more in accordance with the vitality of the centre, than with anything we can do to stimulate it. Hence it is only by very frequently repeating experiments in similar conditions, and by careful interpretation of the results, that conclusions of any cogency can be drawn. Some illustrative experiments may be given.

When a dog whose brain stem had been severed at level 5, was made to breathe into a small closed space (football bladder) the rate though not the height of the gasping was markedly increased (from 8 to $10\frac{1}{2}$ per minute). When oxygen alone was lacking (the animal breathing N_2 through valves), again the gasping became rapid, but to a less degree (9 per minute). In each case the effect took about a minute to come on, and to pass off. The blood-pressure gradually fell when O_2 was diminished, and rose again slightly when air was supplied, but it never exceeded 30 mm. Hg. The experiment indicates, that O_2 lack, even without excess of CO_2, stimulates the gasping centre, an observation strongly supported by a number of other facts. The stimulation was stronger when CO_2 was at the same time in excess, but the more active state of the centre at the beginning of the experiment would to some extent account for this.

In an experiment on a cat whose brain stem was divided between levels 4 and 5 (Fig. 1) the gasping centre was in good condition; here the

addition by continuous ventilation of 12½ p.c. of CO_2 and a further 2 p.c. of O_2 to the 73 p.c. of O_2 previously employed immediately increased the rate of gasping 50 p.c., from 6 to 9 per minute. Later, the response of the centre to various gases was negligible, which emphasises the remarks above made on the care with which conclusions must be drawn. When only 10 p.c. of oxygen was perfused through the lungs, the gasps did not quicken as might be expected, they simply began to fail from the lessening vitality of the centre, which somewhat improved again on 73 p.c. O_2, so that the gasping became more brisk and frequent.

In a cat which died by circulatory failure due to excess of ether, though the heart could not be re-started, yet by constantly massaging it, sufficient circulation was kept going to re-awaken gasping after it had ceased for 13 minutes. At first artificial respiration with the pump and later continuous ventilation with CO_2 18 p.c., O_2 30 p.c. was carried out and gasping continued for half an hour. If during this period the cardiac massage was stopped gasping doubled in rate within 10 seconds and resumed its normal rate the instant massage was recommenced. This effect must have been due to lack of O_2, since CO_2 was in any case in great excess. The experiment also indicates the very rapid response of the gasping centre to changes in the circulation.

Another observation of interest was made on a cat during recovery of respiration after compression of the vertebral arteries, one carotid being left open. Gasping had given place to apneuses with gasps superimposed (the vagi had been cut previously) and later, respiration of normal type had been resumed, but with here and there a gasp superimposed on the inspiration. The animal was next made to breathe my expired air from a large bag (*i.e.* CO_2 5 p.c., O_2 16 p.c.), the gasps increased markedly. Pure oxygen was now supplied, the superimposed gasps ceased within half a minute, leaving the respiration normal (Fig. 2).

The rate of gasping lessens during and after artificial respiration, which confirms the evidence adduced above that the centre is influenced to some extent by the chemical composition of the blood. I have never been able to produce apnœa when the higher respiratory centres were dead. It is found that even when in a fresh and healthy animal, the first cut is made at level 5, gasping occurs instantaneously, although there could have been no great impurity of the blood either in the way of O_2 lack or CO_2 excess. Both these observations point to the gasping centre being when uninhibited either highly automatic or else sensitive to even moderate lack of O_2 and excess of CO_2.

During apneustic respiration when gasps are superimposed on the

apneuses, they can be made to lessen or disappear by supplying plenty of oxygen, and they increase again if oxygen is withheld. Here part of

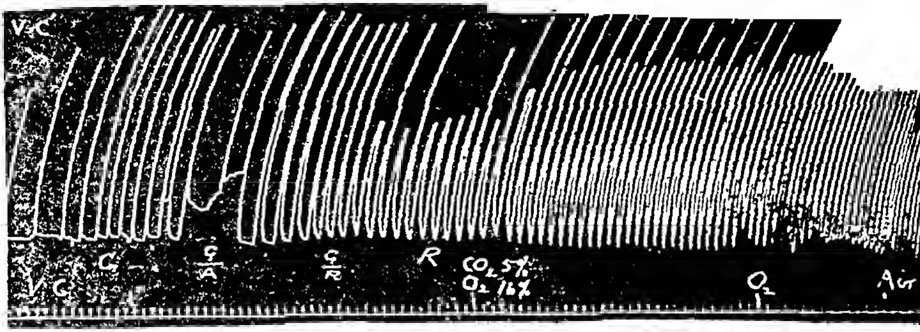

Fig. 2. Cat. In this and the following figures, the upward line is inspiration, and the time tracing 5 sec.

the effect is probably due to the increased or lessened inhibition of gasping by the strengthening or weakening of the apneustic centre as the result of the free supply of oxygen or lack of it. In a particular instance of this sort the blood-pressure remained high, though gradually falling from 200 mm. to 160 mm. The gasps on the apneusis occurred on air breathing at a rate of 5 per minute at first; as oxygenation lessened this rate gradually rose to 8. When pure oxygen was supplied the rate lowered to 6, while when H_2 was breathed the rate rose to 10 and the apneusis ended.

It is known that in man sudden powerful stimulation of almost any afferent nerve, especially if it causes pain, may evoke a typical gasp. Electrical stimuli applied to the nœud vital have a similar effect, so that the above results may depend partly on cerebral inhibition of the higher respiratory centres and partly on direct stimulation of the gasping centre.

The only nerve which, so far as I have seen, has any specific action on gasping is the vagus; the nature of this action is not easy to investigate. Since Section 5 cuts off the main part of the vagal nucleus and its afferent fibres from the gasping centre, we are compelled to judge of the vagal effects in experiments in which a higher section has been made and when it appears from the blood-pressure and type of breathing that the gasping centre alone survives. Out of a great many experiments only half a dozen or so seemed to me at all worthy of consideration. One may instance a cat in which, while pure gasping was occurring, the vagi were frozen, two convulsions occurred and then gasping continued at a

markedly increased rate, during the minute before freezing the rate was 10, in the minute after 16.

In Fig. 3 *c*, a section had been made through the level of the striæ,

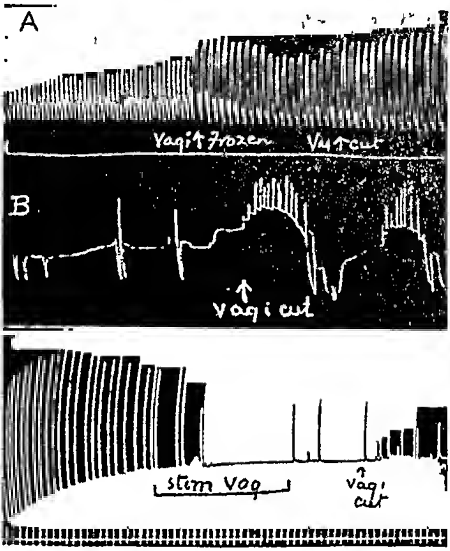

Fig. 3. Cat. Effect of vagotomy during *A*. respiration; *B*. apneusis, *C*. gasping.

a convulsion resulted, and this was followed by pure gasping; stimulation of the vagi very definitely inhibited the gasping (cf. Fig. 4 *c*) subsequent section of the vagi at once stopped this inhibition and gasping recommenced, though somewhat irregularly. In all cases where the higher centres were really dead, inhibition of gasping both in height and rate was the result of moderate vagal stimulation if it had any effect at all. On the other hand, if the apneustic centre was still alive and was inhibiting gasping, then vagal stimulation by inhibiting this centre released gasps, *i.e.* by inhibition of an inhibition (Fig. 4 *b*).

If the vagal stimulation lowers the blood-pressure, this also will tend to increase gasping, just as stopping cardiac massage did in the experiment described above at p. 83.

In confirmation of the releasing effect of vagotomy on gasping is the fact that if during an apneusis, the vagi are cut, the effect is generally

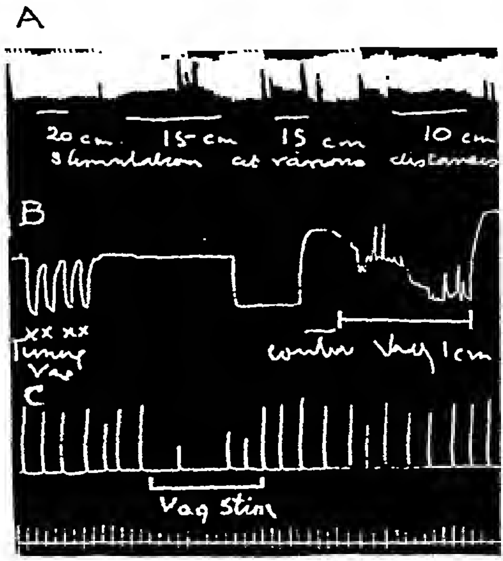

Fig. 4. Cat. Effect of vagal stimulation during *A*. respiration; *B*. apneusis; *C*. gasping.

to heighten and lengthen the apneuses and to release gasps upon them (Fig. 3 *b*). The factors which may conduce towards this result are the direct effect of vagotomy on the gasping centre above mentioned and the increased chemical stimulation of gasping due to the prolongation of the apneusis. It is also a possibility that it is in response to vagal messages that the apneustic centre normally inhibits gasping. Indeed, whenever apneuses have gasps superimposed on them at regular intervals it may be concluded that either the vagi have been cut or that they are more or less out of action for the time being.

It appears then that vagal impulses when acting directly on the gasping centre tend to inhibit gasps, while vagotomy releases them. As long as the apneustic centre is alive, however, these direct effects are obscured by the facts that the vagus acts most powerfully on the apneustic and expiratory centres and that the former of these has a stronger direct effect on gasping than the vagus has. Further, chemical stimulation of the gasping centre is more effective than vagal stimulation of it. Hence in the intact animal vagal stimulation may release gasps in spite of the fact that the direct specific effect of vagal stimulation on the gasping centre is inhibitory.

Apneustic respiration and expiration. In Fig. 5 it will be noticed that when a rabbit was made to breathe CO_2 14 p.c. O_2 28 p.c. the succeeding

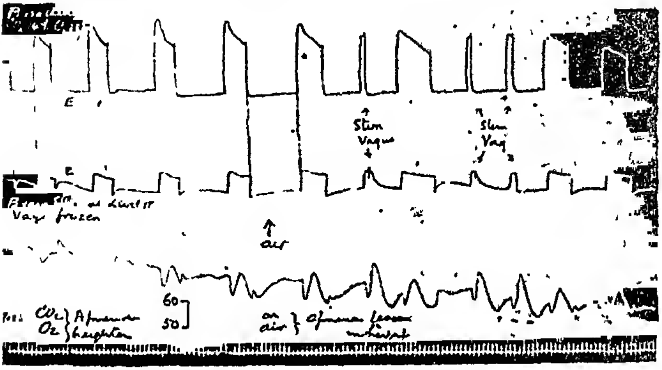

Fig. 5. Rabbit. Effect of breathing excess of CO_2 during apneustic respiration. Upper tracing from thorax, middle from abdomen. Lowest tracing = blood-pressure.

apneuses heightened considerably above the base line (about 25 p.c. increase) and when air breathing was resumed the apneuses diminished again gradually, the length of the apneuses was not much affected. Another noticeable effect was that the expiratory spasms increased markedly in intensity and slightly in duration (see Fig. 2 of my second paper(1)). This and many similar experiments indicate that excess of CO_2 increases the excitability of (stimulates) both the apneustic and the expiration centres; it does not materially influence the duration of the apneusis. That variation in the duration of apneusis and not of expiration is the factor which chiefly determines the rhythm of breathing is shown very clearly in Fig. 6 from the same rabbit. Here vagal stimulation during either phase at once caused its inhibition, but while by periodical stimulation as soon as apneusis was resumed (timing expiration) the whole cycle of movements could be accelerated so that a very good copy of normal respiration could be produced, periodical stimulation as soon as expiration had occurred (timing inspiration) merely eliminated the prolonged expiratory spasms; it did not accelerate the whole cycle, and the result was a series of prolonged apneuses with only momentary pauses between them. These observations confirm the view that apneusis is the natural tonic position, periodical inhibition of which produces normal respiration. The

active expiratory spasms are not tonic but tetanic; they are incidental and are not the basis upon which respiration is normally built up.

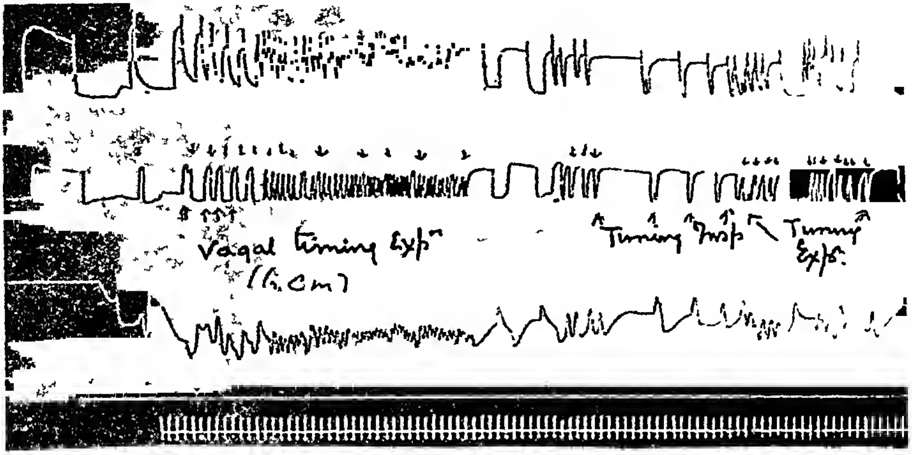

Fig. 6. Continuation of Fig. 5. Effect of vagal stimulation during apneustic respiration.

The increase of the height of apneuses and depth of expiration due to CO_2 excess is important since it explains the increased extent of the respiratory movements which occurs when CO_2 is present in too great amount during breathing of normal type.

If during apneustic respiration oxygenation of the blood is slightly or moderately diminished, for instance when air is breathed through valves instead of pure O_2, there is an increase in height and a decrease in length of apneuses which disappear when the pure O_2 is given again. The outstanding effect of marked or intense oxygen lack is however due

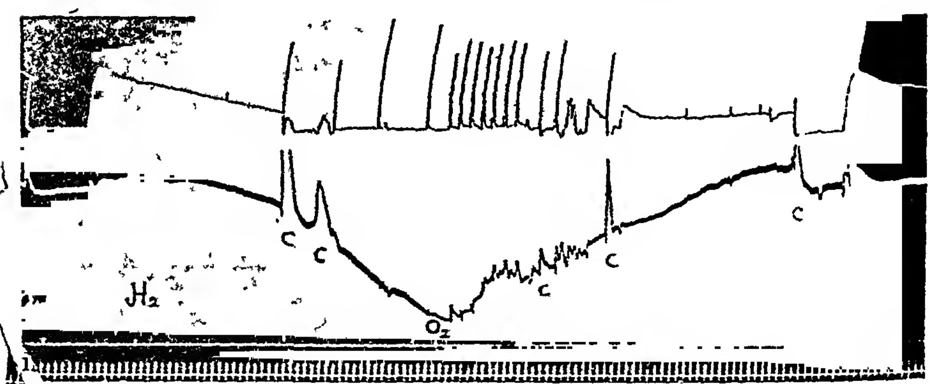

Fig. 7. Cat. Failure and revival of apneusis. Upper tracing from thorax. Lower tracing = blood-pressure.

to the resulting damage to the vitality of the centre. Fig. 7 shows how when H_2 was breathed the inspiratory tonus failed, giving place first to short feeble apneuses and soon convulsive expiratory spasms, and finally to gasps, the blood-pressure sank rapidly and death was only prevented by supplying O_2 again, when recovery took place in the reverse order.

The only effect of O_2 lack on the expiratory centre is to lower its vitality, it has no stimulant effect on expiration.

I do not propose to review the very extensive literature on the effect of the vagus. It is sufficient to state that the views of Hering and Breuer (2), supported by Head (3) and others, are those generally accepted, namely that the vagi contain both inspiratory and expiratory fibres, stimulation of which gives rise to inspiration and expiration respectively. With the view so expressed I differ because I regard, as Gad (4) did, the afferent vagus as essentially inhibitory in its effects on respiration.

CO_2 appears to increase the excitability of all the respiratory centres, and if, during breathing of apneustic type, it is administered with enough O_2 to preserve the vitality of the centres, it stimulates both inspiration and expiration, increasing the height of apneusis and the depth of expiration. It seems likely, therefore, that if the vagi contained excitatory fibres for both inspiration and expiration, continuous stimulation of it would produce the same results as administration of excess of CO_2. This is not at all the case; momentary (or periodically repeated) stimulation of the vagi definitely cuts short (inhibits) both the inspiratory and expiratory phases of apneustic respiration; thus accelerating the rhythm but diminishing the amplitude of the respiratory movements (Figs. 4 *a* and 6). Excess of CO_2 has just the reverse effect—increasing the amplitude and if anything slowing the rhythm of this type of breathing.

Continuous stimulation of the vagus produces inhibition of apneustic phenomena, and since the tonic phase of this type of respiration is inspiratory, the vagal arrest shows itself in apnœa at the base line level. To obtain such an effect, it is necessary to employ continuous ventilation, otherwise the chemical calls become so strong as to overcome the vagal inhibition and short apneuses or gasps appear (Fig. 4 *b*).

The only conclusion which seems possible is that, while CO_2 acts by exciting the respiratory centres, the vagus has the reverse action and inhibits such of them as it acts upon.

Vagotomy during apneustic respiration (Fig. 3 *b*) lengthens and heightens apneusis and releases gasps so that they become superimposed on the inspiratory tonus. From a ventilation point of view this is

advantageous, so that while an apneusis without gasps lasts in the absence of continuous ventilation only for one to three minutes, an apneusis with gasps superadded was in one instance continued, for 13 minutes, and, indeed, it only ceased when H_2 instead of O_2 was supplied. This does not indicate that vagal control of apneusis and gasping is a retrograde function, but rather that it is only a transitional stage towards respiration of normal type which in the unnatural conditions under consideration does not happen to be advantageous.

Vagotomy also releases and lengthens the expiratory phase of apneustic breathing, and expiration becomes spastic until the centre tires, as it generally does in five or ten minutes.

If during apneusis the abdominal or thoracic parietes or contents are interfered with or irritated, inhibition and expiration result, and expiration may be similarly inhibited. This observation raises the possibility that deep sensibility has considerable respiratory importance. The trigeminus has no tonic action on respiration. If stimulated momentarily by blowing into, or irritation of, the nostrils or by electrical stimulation of the nerve or its cut cerebral end, the effect is immediate inhibition of apneusis. If the stimulus is intense or is continued for some time sneezing may result. Irritant gases if passed through the nose cause arrest of breathing till the chemical calls for aeration become irresistible. Section of the trigemini does not affect respiration if managed without injury to the pons.

Conclusions.

The factors which regulate the three lower respiratory centres are partly chemical (lack of O_2 and excess of CO_2) and partly nervous (vagal and trigeminal impulses mainly).

(1) The gasping centre is stimulated by lack of O_2, by excess of CO_2, and stimulation is most powerful when both these factors coexist. The specific effect of vagal impulses on this centre is inhibitory. Yet vagal stimulation may release gasps on certain occasions by inhibiting the apneustic centre's tonic inhibition of the gasping centre. Vagotomy invariably tends to liberate gasps.

(2) The expiratory centre is stimulated by excess of CO_2. Oxygen lack has no effect on it except to impair its vitality. Vagal stimulation inhibits expiration while vagotomy releases and prolongs active expiratory movements.

(3) The apneustic centre is stimulated by excess of CO_2 and much less effectively by moderate lack of O_2. Severe lack of O_2 causes the tonic apneusis to fail and hence indirectly as well as directly liberates gasping.

Vagal stimulation inhibits apneusis as it does expiration. Thus, during either phase of the apneustic type of respiration, vagal stimulation inhibits the existing phase and releases the reverse phase. In this way vagal impulses accelerate the rhythm of respiration and diminish the extent of the respiratory movements in both directions This is a tonic effect and hence vagotomy invariably slows and deepens respiration

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AN ERGOMETER ADAPTABLE FOR EITHER HAND- OR FOOT-MOVEMENTS. By E. P. CATHCART, G. M. WISHART and J. McCALL.

(*From the Institute of Physiology, Glasgow University.*)

The varieties of apparatus which have been devised for the estimation of external work done are many, ranging from the simple hand dynamometer of Collin through the various forms of ergograph originated by Mosso to the bicycle type of ergometer of Atwater and Benedict, Krogh and Martin and the still more complicated apparatus of Johansson. The simplest of the more complicated types of ergometer, which alone suffice for the determination of the energy expenditure during the performance of severe or prolonged spells of work, involving large groups of muscles, is the bicycle of Martin where, in place of the electromagnetic resistance utilised by Benedict and by Krogh, a band frictional resistance connected with spring balances is employed. In the Johansson type, which, in contradistinction to the bicycle form, is used for arm movements, the work done is that of raising a variable weight through a given distance by pulling with both hands on a suitable handle. This apparatus has the particular merit that it serves for carrying out both negative and static "work" experiments as well as those with ordinary positive work.

It was found in the course of a series of experiments, in which it was desired to vary the work done both as regards amount and rate in a given time and also to compare the arms and legs as effective agents in the production of external work, that none of the older patterns of apparatus sufficed. The apparatus now about to be described (see Fig. 1), which allows of wide variation in effort, was designed. A steel wheel turned all over measuring 1·5 metres in circumference by 2·5 cm. across the face and weighing approximately 22·7 kilo. is mounted on an ordinary rear wheel bicycle hub. This wheel is fitted on a heavy wooden frame 1·7 m. long at the base made of well seasoned pine 8 cm. square with a cross supporting piece at the wheel end 1 metre long. On this floor-frame is built a rigid table 66 cm. long, 23 cm. broad and 57 cm. high. Fixed at the end of this table is a cast iron bracket in which are housed two heavy Hofmann ball bearings to carry the crankshaft. The cranks

are overhung and each has a slot 13 cm. long along which is fixed a millimetre scale so that the crank pins can be adjusted to any required stroke.

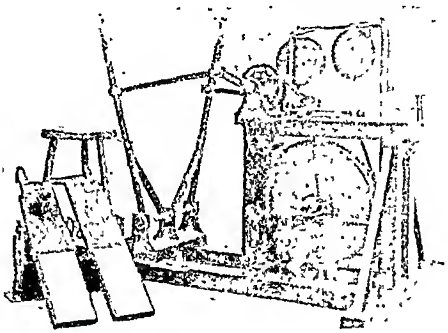

Fig. 1.

The maximum throw is 14 cm. giving a stroke of 28 cm. Attached to the crank shaft by a hub which slides along the shaft on a long key there are three driving sprockets of varying diameters which give three different gear ratios of 1:1, 1:1·7 and 1:2·4. The mobile hub permits of any one of the three sprockets being brought into line with the sprocket on the fly-wheel hub, and when in position the hub is fixed to the crank shaft by means of a screw pin. The drive is through a roller chain which, in order to allow for the variation in the size of the crank shaft sprocket and so to maintain constant tension, passes over a tension sprocket mounted on ball bearings. This tension sprocket slides in a slotted angle plate fixed on the table and can be set at any desired place. In order to ensure that the chain tension is the same in each experiment the whip of the chain, on application of a standard force (weight of 10 lbs.) at a definite point on the chain, is adjusted to a fixed amount. The type of gearing described was adopted so that the frictional losses with all gears would be approximately the same. On the top of the table is erected a rectangular parallel steel frame between which is pivoted a steel arm to which are suspended two sensitive 5 kilo. spring balances with 19 cm. dials graduated in 20 grm. A piece of cord is attached to each balance and guided over two ball bearing aluminium pulleys to each end of the brake belt. The brake belt consists of a length of fine balata belting 125 cm. long and 4 cm. broad, rendered pliable by treatment with vase-

another and each separately connected to the 250-volt lighting supply. In the field circuit was placed an ammeter and in the armature circuit an ammeter and voltmeter.

The ergometer, by controlling the resistances of the motor, was adjusted to run at a certain speed as registered by the tachometer and, when running steadily at the selected speed, readings were taken of the voltage and current in the armature circuit. The driving belt from motor to ergometer was then removed and the belt of a very small spring dynamometer (registering loads up to 500 grm. and reading in 10 grm.) was applied to the pulley of the motor shaft. By adjustment of the tension of the dynamometer band the former speed, armature current and voltage were exactly reproduced and when this was attained the dynamometer readings were noted. The current passing round the field coils was maintained at a constant value by the adjustment of a variable resistance in the field circuit using the field circuit ammeter as indicator.

The results obtained (in large calories per min.) are given in Table I and represent the energy expended in driving the machine unloaded at four different speeds with each of the operating mechanisms (levers or treadles) in position and under varying conditions of stroke and gear.

TABLE I.

Stroke (i.e. distance of connecting rod from centre of crankshaft) cm.	Operating mechanism	Gear	Revolutions per minute of ergometer flywheel 163 cals.	117 cals.	71 cals.	31 cals.
14	Hand-levers	High	·0573	·0329	·0175	·0065
10	,,	,,	·0573	·0329	·0175	·0065
5	,,	,,	·0544	·0329	·0175	·0065
14	,,	Mid	·0573	·0329	·0142	·0055
10	,,	,,	·0573	·0350	·0150	·0065
5	,,	,,	·0516	·0329	·0150	·0055
14	,,	Low	—	·1275	·0325	·0109
10	,,	,,	—	·0741	·0275	·0104
5	,,	,,	—	·0391	·0225	·0087
14	Large treadles	High	·0573	·0370	·0125	·0049
10	,,	,,	·0545	·0329	·0150	·0049
5	,,	,,	·0516	·0247	·0125	·0044
14	,,	Mid	·0745	·0411	·0200	·0076
10	,,	,,	·0688	·0370	·0187	·0065
5	,,	,,	·0688	·0329	·0175	·0065
14	,,	Low	—	·1070	·0300	·0109
10	,,	,,	—	·0699	·0225	·0098
5	,,	,,	·0860	·0535	·0225	·0087
4·4	Small treadles	High	·0487	·0350	·0175	·0060
4·4	,,	Mid	·0516	·0288	·0175	·0055
4·4	,,	Low	·0802	·0494	·0275	·0120

It will be remembered from the construction of the apparatus that the higher the gear the fewer per flywheel revolution are the reciprocating movements of the hand levers or treadles.

It will be observed from the tabulated results that in all cases the energy expenditure in driving the machine unloaded is negligible in comparison with the energy output of the subject; a slight error either in the calibration or in the setting of the spring balances of the ergometer would result in a value being obtained for the work done which would differ from the true work by an amount very much greater than any of the values given in the above table. Further, the highest value recorded for energy losses in the machine 0·1275 cal. per min. was found under conditions which on account of the length of stroke and rapidity of movement would be impossible of human performance for other than the most limited periods.

It will also be noted that as the gear ratio is lowered and consequently the speed of the reciprocating parts increased there is a general tendency for the energy required to drive the machine to rise. This is most noticeable in the case of the hand levers probably on account of their greater mass.

The greater part of the expense incurred was defrayed by a grant from the Royal Society Government Grant Committee.

NERVE REGENERATION FROM ONE INTO THE OTHER OF TWO RATS UNITED IN SIAMESE PAIRS[1]. By B. MORPURGO.

(From the Institute of General Pathology, University of Turin.)

If two rats are united parabiotically no nervous connexions are formed between them. The following experiments have been carried out in order to verify if this was due to the incapacity of the nerves of one individual to grow into the other. For this purpose artificial nervous connections were made in Siamese pairs of rats.

The cut end of the proximal stump of the right sciatic nerve of the left-hand rat was brought into apposition, with the cut end of the distal stump of the left sciatic nerve belonging to the right-hand rat, whilst the proximal portion of this nerve was torn away from its roots. Out of 38 operated pairs 19 supplied suitable material for study. Of these pairs six are still alive, the others have been utilized for histological research on the regeneration of the nerve at different stages between the 7th and the 374th day. The specimens of nerves were prepared by Cajal's reduced silver method, whilst those of nerve-endings in the muscles were impregnated by Ruffini's gold-chloride method. On the 7th day the connected nerve stumps were surrounded by recent granulation tissue. Many newly formed fibres were seen growing out from the axis-cylinders of the central stump. They had nearly reached the proximal end of the peripheral stump, partly wandering into the granulation tissue. This picture is exactly the same as that of the regeneration of a nerve in a single rat. Already on the 9th day, neurotization of the peripheral stump had started: some very fine filaments, originated from the central stump, had penetrated nearly as far as $\frac{1}{2}$ mm. into the peripheral stump. At the succeeding stages the newly formed fibres had advanced in the various pairs of rats more or less rapidly. On about the 20th day they had attained the cavus poplitaeus. Between the 45th and the 60th day they had reached the periphery, where they formed new end-organs.

Later on the nerve fibres arrange themselves into bundles and some of them increase in size. From the gluteal branches of the left-hand rat,

[1] Living animals, specimens and drawings regarding the subject were shown at the International Congress of Physiology, Edinburgh, July 23–28, 1923.

which were cut in order to prepare the proximal stump of the sciatic nerve and to bring it into apposition with the distal stump of the right-hand rat, new fibres sprout out and grow along the sciatic furrow. Little by little these fibres reach the stumps of the tibial and peronacus nerves of the left-hand rat. Therefore the sciatic trunk of the left-hand rat sends branches both into the leg of the right-hand rat and into that of the left-hand one; a nerve path in the shape of a Y turned upside down is formed which, from the legs of both rats, reaches the spinal cord of the left-hand rat. The regeneration of the nerve-endings takes place in both rats. The regenerated motor-plates are superabundant and irregular; many muscle-fibres possess three or even more plates arising either from different nerve-fibres or from branches of the same fibre or from secondary fibres arising from a plate. There are frequently manifold and complicated anastomoses between plates belonging to the same or to different muscle-fibres and even end-plates have been observed which were attached half to one muscle-fibre and half to an adjacent one. There are many large and many small plates. Muscle-spindles and neuro-tendinous organs also regenerate; terminal expansions in these last organs are imperfect and sometimes altogether missing.

In unequal Siamese pairs of rats the activity of nerve regeneration depends upon the nutrition of the organism which supplies the regenerating fibres and is not remarkably influenced by the nutrition of the organism in which the newly formed fibres penetrate.

Physiological experiments show that after one month and a half motility, sensibility and reflexes appear again in both legs; the contraction of the muscles in the leg of the right-hand rat can be caused by stimulating the sciatic nervo of the left-hand rat near the issue of the nerve from the pelvis; after two months and a half crossed reflexes also appear; by exciting the sole of the foot of one rat contractions of muscles innervated by the sciatic nerve belonging to the other rat are caused: at the third month crossed reflexes are obtained from the left-hand rat in the right-hand one, by stimulating the root of the tail, the scrotum and also cephalic portions such as the ear.

It has not yet been possible to prove that movements of the leg of the right-hand rat are accomplished by the will of the left-hand one. Long after the operation there is a severe atrophy of the muscles of the legs and especially of those of the right-hand rat, very likely from lack of use.

If only one of the principal branches of the sciatic nerve, for instance the poplitaeus or tibialis, belonging to the left-hand rat was cut and con-

nected with the whole peripheral stump of the sciatic nerve of the right-hand rat, the crossed reflexes from this to the other rat appear at the same time as the reflexes in the same individual, whilst those from the left-hand to the right-hand rat appear one month later. The reflex arc for reflexes in the same individual is re-established sooner than the arc for those crossed reflexes which involve a regenerated motor path and at the same time as that for those crossed reflexes which involve an undamaged motor path.

A COMPARISON BETWEEN THE COLORIMETRIC AND THE ELECTROMETRIC METHODS OF DETERMINING THE HYDROGEN ION CONCENTRATION OF BLOOD.

By RUTH CONWAY-VERNEY, M.R.C.P. and
L. E. BAYLISS, B.A., Michael Foster Student.

(From the Physiological Laboratory, Cambridge.)

Evans(5) found that the hydrogen ion concentration of blood and sodium bicarbonate solution as determined by the dialysis method of Dale and Evans(3) was pH 0·2 greater than that of the same solution under the same conditions as determined by the hydrogen electrode. After excluding various possible explanations such as loss of CO_2 during dialysis, he came to the conclusion that the error lay in the hydrogen electrode, and that the platinum black on it catalyses a reduction of the CO_2 present in tho gas atmosphere to formic acid. In view of the fact that the hydrogen electrodo is usually regarded as the standard in all measurements of hydrogen ion concentration, it seemed desirable to repeat Evans' experiments.

Several workers have already published experiments on this problem, notably Cullen and Hastings(2) who found a perfect agreement between indicator and electrode in the case of bicarbonate solutions; they did not perform any experiments on blood. Warburg(11) points out that Evans' explanation of his discrepancy is wrong, since his values of pK_1 (the constant in the Henderson-Hasselbalch equation) are not dependent on the CO_2 tension. He has found, moreover, that the combined CO_2 in an alkali bicarbonate solution does not decrease when the solution is treated for half an hour or more with hydrogen in the presence of platinum black. These, together with other reasons, led Warburg to the conclusion that Evans allowed the hydrogen in contact with his electrode to become contaminated with oxygen. Indeed, Evans admits in the paper referred to, that sometimes his gas contained oxygen; and the technique which he used would make it very difficult to avoid this.

Cullen and Hastings(2), and Chambers(1), on the contrary, ascribe the discrepancies observed in the determination of the cH of *blood* to the unequal distribution of ions across the dialysis membrane, apparently regardless of the fact that this phenomenon can be ruled out as of

no importance both (*a*) from the fact that the same discrepancy was observed by Evans in bicarbonate solutions, where no dialysis was performed, as in blood which was dialysed, and (*b*) by calculation from the thermodynamic expression derived by Donnan(4) which shows that the difference in *c*H across the membrane is far smaller than that to be accounted for. Taking the indiffusible (protein) ion concentration as $M/100$ and the diffusible ion concentration as $M/10$, the distribution ratio of diffusible ions between the outside and the inside of the membrane is 1·1: or, if the *p*H outside is 7·00, it will be 6·96 inside. This differencc is hardly, if at all, outside the limits of experimental error even in the most advantageous circumstances, and the true value is almost certainly smaller than this.

A direct experimental proof of the unimportance of the "Donnan effect" in this connection has recently been published by Taylor(10), who measured the potential difference across the membrane during dialysis. It is clear from his observations that no P.D.'s greater than a few millivolts exist across the membrane unless it has become stained with hæmoglobin. As A. V. Hill(6) has shown, there can be no difference in ion concentration across a membrane without a corresponding potential difference, whether it arises from a Donnan equilibrium or in any other way.

Technique. Since it is essential that the blood exposed to the electrode should be completely reduced, while that in the dialysis membrane is unavoidably completely oxidised, unless special, rather complicated methods are employed, we came to the conclusion that it would be best to work the two experimental methods more or less independently. The general procedure was, therefore, to saturate a sample of sheep's blood (with 1 c.c. 0·5 p.c. sodium fluoride added to 10 c.c. blood at the time of shedding) in a tonometer with air containing a known tension of CO_2. This latter was estimated by a sample of gas taken from the tonometer after the removal of the blood, in case any CO_2 taken up by or lost from the blood should have altered the original tension, the CO_2 pressure required, of course, being that after equilibrium had been reached. A second sample was reduced *in vacuo* and saturated with hydrogen containing a known tension of CO_2 in the electrode vessel; the first lot was used for the colorimetric estimation and the second for the electrometric. In this way a $c\mathrm{H} - CO_2$ tension curve was built up containing both colorimetric and electrometric points. If the one straight line satisfied both sets of points, after the necessary corrections had been made, the agreement between them is vindicated.

A. Electrometric. An ordinary set-up was used consisting of a Clark electrode vessel, a saturated KCl bridge, and a saturated KCl calomel half-cell. This whole set-up was placed inside a constant temperature air-bath, and was standardised by means of Michaelis' (7) standard acetate using the values given by him for the potential against a saturated calomel half-cell. The hydrogen was generated electrolytically, passed over saturated potash, and, at first over a wire kept incandescent electrically. No difference could be detected, however, in the values obtained for the potentials whether the wire was glowing or not, so its use was discontinued. In view of the extreme importance of traces of oxygen and the possibilities of leakage in the apparatus the glowing wire has been reinstated.

The CO_2 was added to the hydrogen in a large aspirator over saturated calcium chloride, a sample taken during the saturation of the blood in the electrode vessel and analysed in a Haldane apparatus. To save time and to avoid as far as possible bringing the electrode into contact with oxygen, the blood was given a preliminary reduction *in vacuo.* 9 p.c. was added to the *c*H readings so obtained ($= -0{\cdot}04$ *p*H) in order to correct for the change produced by reduction of the blood (Parsons (8)).

It occurred to us that we should avoid errors which might possibly arise by comparing reduced blood in the electrode vessel with oxygenated blood in the dialysis membrane, by using CO blood. The results obtained, however, were very acid and at first we attributed this to an effect of the CO on the electrode causing it to be depolarised; this has since been shown to be incorrect, and the errors were found to be due to the presence of about 3 p.c. of oxygen in our sample of CO.

B. Colorimetric. The method used for these determinations was substantially that described by Dale and Lovatt Evans (3). The apparatus comprised a semi-permeable membrane attached to a vulcanite top which is corked immediately after the blood is run in to prevent loss of CO_2. The blood is dialysed against a 0·9 p.c. saline solution contained in a hard glass comparator vessel with a ground glass flat bottom which can be used in a Dubosc colorimeter. Since it has been shown by Taylor (10) that a permanent potential difference of about 10 millivolts may be obtained with membranes stained with hæmoglobin, or used too often, our membranes were soaked for at least 48 hours in 50 p.c. alcohol before filling with blood. They were soaked in alcohol for a week more after testing for leaks and permeability, and washed thoroughly with distilled water. Four, five or six membranes were filled directly from the tonometers in which the blood had been equilibrated at various CO_2

tensions, and the mean of these dialysates taken. It was found that the readings varied to a lesser degree if dialysis was allowed to proceed for at least 30 minutes, not 15 as originally suggested, and were frequently identical. 0·02 p.c. neutral red, 0·02 p.c. phenol red and 0·02 p.c. cresol red were all used as indicators and the amount (six drops) was strictly measured as variations in this were found to cause distinct differences in colour and a corresponding error in amounts of standard *p*H solution used in titrations. Control observations where all three indicators were used on the same solution gave readings ·02 to ·04 *p*H more alkaline with neutral red, ·02 *p*H more alkaline with phenol red, and agreement in nearly every case with cresol red, when compared with the electrometric readings. In later observations, therefore, cresol red was used.

Corrections for the salt error of the indicator were not made in these observations as ·05 p.c. of sodium fluoride was the only salt added to the blood and the values to be used in such relatively weak solutions are by no means generally agreed upon. The solutions used as standards were the phosphates recommended by Sörensen (9) and given by Dale and Evans (3); they were checked by the hydrogen electrode before being used.

As already stated, it was not possible in the majority of cases to compare readings on blood equilibrated at the same tension of CO_2. In the few cases recorded in Table II a sample of blood was run directly from the electrode vessel into a dialysing membrane, after the electrometric determinations had been made. It was never possible to fill more than one membrane, however, so the agreement between the electrometric and the colorimetric readings may be regarded as good, except in the case of the CO blood, in which case the discrepancies have been accounted for.

Results. These are recorded in the figures and some typical results given in the tables at the end.

The agreement between the electrometric and the colorimetric methods is all that could be desired in the case of the ·02 *M* sodium bicarbonate solution, but the values obtained for blood show rather greater variations. It can readily be seen, however, that the points given by the electrode are well interspersed among those given by the indicator and that there is not the systematic difference between them found by Evans.

In order to make the drawing of the mean line through the points on Fig. 2 easier and to demonstrate our results in tabular form, we have

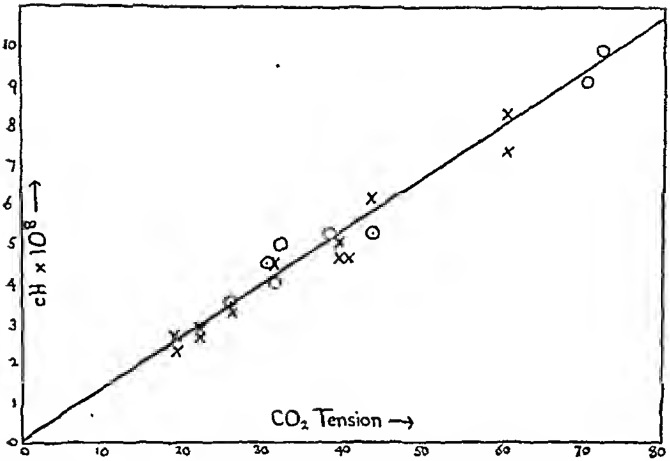

Fig. 1. cH – CO_2 tension curve of 0·02 mol. sodium bicarbonate solution. The colorimetric readings are indicated by crosses and the electrometric by circles.

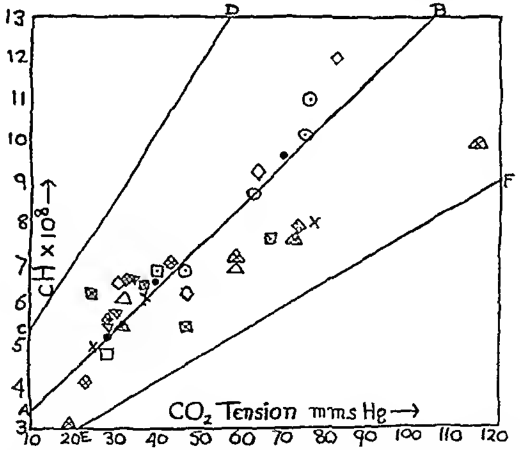

Fig. 2. cH – CO_2 tension curve of sheep's blood. The colorimetric readings are indicated by crosses and the electrometric by dots; the shape of the figure around the crosses and dots indicates the specimen of blood used, as follows:

Sample $A = \triangle$. $B = \nabla$. $C = \square$ $D = \lozenge$. $E = \bigcirc$. Means $= \bullet$ and ×.

ON A POSSIBLE RELATION BETWEEN THE PANCREAS AND THE PARATHYROIDS.

By L. B. WINTER and W. SMITH.

(From the Biochemical Laboratory, Cambridge.)

It is known that removal of one or more of the parathyroid glands from animals results in a lowering of the tolerance for carbohydrate. Total parathyroidectomy results in a condition of tetany, which is relieved by injection of calcium salts, or administration of parathyroid extract. The mechanism by which the parathyroids influence carbohydrate metabolism is unknown. The fact that adrenaline and thyroid extract have been found to raise the blood sugar of rabbits in such a manner as to maintain the normal ratio, copper reducing to polarimetric value suggested that the parathyroids might exercise some function on the carbohydrate metabolism in conjunction with another ductless gland. In the following experiments parathyroid tablets 1/10 grain (Parke Davis) were employed. Rabbits were used throughout.

The possible effect of injection of parathyroid extract on the blood sugar of normal animals was first studied. The injections were given either subcutaneously or intravenously. Three to four tablets, depending on the size of the animal, were ground up in normal saline, lightly centrifuged, and the supernatant fluid injected. Blood sugar determinations were made by Bang's old method. Injection of parathyroid alone was found to be without appreciable effect on the blood sugar. It was found, however, that injection of parathyroid extract into animals suffering from hypoglycæmic convulsions due to insulin, had a very marked effect. The animals quickly became rigid and died in a few minutes. We have previously found that animals can remain in a hypoglycæmic condition, with intermittent convulsions, for a considerable period, and then be recovered with the aid of glucose. It seemed ... that the action of the parathyroid extract was to augment that of insulin. It was possible that a very much smaller dose of insulin ... produce convulsions in a rabbit to which an injection of parathyroid extract had been previously given. The following experiments ... formed to test this point. The same batch of crude insulin

was used throughout. For another purpose it had previously been necessary to cause convulsions in a large number of rabbits. The normal dose necessary to cause convulsions in a rabbit of 2 kilos. in two to three hours had been found to be between 30 and 40 mg. Less than 30 mg. of this sample had never caused convulsions in a healthy rabbit of a similar weight—starved for 24 hours. This period of starvation before injection is a routine procedure. An extract of three to four parathyroid tablets in normal saline was injected subcutaneously or intravenously. Ten minutes later 10 mg. of insulin was injected subcutaneously or intravenously, depending on the method employed for the parathyroid. With intravenous injection of both gland extracts convulsions occurred ery rapidly. The effect was slower after subcutaneous injection of rathyroid and insulin, but convulsions were readily induced following s small amount of insulin. Control experiments showed that 10 mg. nsulin alone had only a moderate effect on the blood sugar, as would xpected from our previous results. When convulsions were caused by ction of parathyroid and insulin complete recovery always occurred owing subcutaneous injection of glucose. Recovery appeared to be cker than in the case of those animals which had been convulsed by ulin alone (30–40 mg.). This is perhaps to be expected since there is such an excess of insulin remaining in the animal when convulsions ur. The effect of parathyroid extract on the ratio of copper reducing olarimetric value of the blood sugar was studied, but no ratio outside he variation normally met with in the rabbit was observed.

Summary.

After a preliminary injection of parathyroid extract into rabbits, convulsions are induced following 1/3 to 1/4 the normal dose of insulin.

We wish to thank the Department of Scientific and Industrial Research (W.S.) and the Medical Research Council (L.B.W.) for personal grants held during the course of this work.

Some typical protocols are appended.

PROTOCOLS.

R. 3·0 kg.	Time	Blood sugar %	R. 2·1 kg.	Time	Blood sugar %
	10·30	·10		10·15	·11
Injected with four parathyroid tablets	10·45	—	Injected with 10 mg. insulin	10·30	—
	12·30	·10		12·00	·09
	3·00	·11		1·15	·08
	5·00	·11		3·00	·10
				4·30	·10

R. 2·2 kg.	Time	Blood sugar %	R. 2·5 kg.	Time	Blood sugar %
	10·30	·12		10·00	·09
Injected with three parathyroid tablets	10·50	—	Four parathyroid tablets	10·15	—
10 mg. insulin	11·00	—	10 mg. insulin	10·25	—
	12·00	·07		11·30	·05
	12·30	Convulsions ·05		12·00	Convulsions ·04
Glucose injected	12·45	—		12·10	Glucose inj.
Animal eating	12·55	—		12·15	Eating
	2·30	·14		1·15	·09
	6·00	·12		2·30	·11

	Polarimetric readings on successive days (R.)	Copper reducing value (R.)
Rabbit, three tablets parathyroid intravenously, killed 10′ later (47 c.c. blood)	·01, ·04, ·05, ·07	·07
Rabbit, three tablets parathyroid subcutaneously, killed 15′ later (67 c.c. blood)	·08, ·10, ·11, ·11	·11

THE REGULATION OF RESPIRATION. Part II. Normal Type. By THOMAS LUMSDEN, M.D. (Aberd.).

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The generally accepted view is that, as insisted on by Haldane and his co-workers (1), CO_2 and possibly other fatigue products, *e.g.* sarcolactic acid, are the normal stimulants of the respiratory centres. Thus a rise of 0·2 p.c. of CO_2 in the alveolar air, doubles the pulmonary ventilation, while O_2 lack does not increase the respiratory rate until the atmospheric O_2 falls below 13 p.c. With regard to the influence of the vagus, opinions vary somewhat, but most authorities favour Head's conclusions (2) that the vagi contain two sets of fibres, one increasing the activity of the inspiratory part and the other increasing the activity of the expiratory part of the respiratory centre. Head confirmed Hering and Breuer's view that dilation of the lungs, however produced, promoted expiration, while whatever caused contraction of the lungs inhibited expiration and produced inspiration. F. H. Scott (3) stated that after vagotomy excess of CO_2 or great diminution of O_2 increases the depth but not the rate of breathing. Probably all physiologists agree that vagotomy invariably causes the breathing to become deeper and slower. Everything I have noticed has confirmed the view supported by Haldane and others, that the amount of CO_2 in the blood is the normal respiratory stimulus.

In previous communications (4) I have put forward evidence to indicate that natural quiet breathing results from periodic inhibition of prolonged inspiratory tonus, through the rhythmical activity of the pneumotaxic centre in the upper half of the pons. My view is that the excitability of this centre is raised by the H-ions circulating through it, till it reaches a certain point at which discharge occurs, giving rise to an impulse which inhibits momentarily the inspiratory tonus (apneusis). The moment after discharge the excitability of the pneumotaxic centre may be supposed to be at its lowest level and it rises under normal conditions more or less quickly in accordance with the amount of CO_2 in the blood till the next discharge occurs. In accordance with this view the rate of respiration depends primarily on the pneumotaxic centre while the amplitude of the breathing, *i.e.* the amount of air taken in

and expelled at each breath, depends on the stimulation of the apneustic and expiratory centres which both respond as indicated in my previous paper to the percentage of CO_2 in the blood, while the apneustic centre alone is stimulated also to some extent by serious lack of O_2 (10 p.c. or less). It will be apparent that if these views are correct, then even after vagotomy, excess of CO_2 should be able by its effect on the pneumotaxic centre to increase the respiratory rate. The methods employed were those described in my previous papers. As a routine method of recording respiratory tracings I prefer the use of abdominal and thoracic tambours to the diaphragm slip method. A good deal of the effects seen when using Head's method are I think adventitious, and misleading.

Since I have shown(4) that apart from the vagi the rhythm of breathing depends chiefly upon the pneumotaxic centre, Scott's conclusion referred to above would point to that centre being, as far as acceleration is concerned, unresponsive to the amount of CO_2 which circulates through it. In a series of experiments I investigated this point before and after vagotomy, in intact anæsthetised animals (cats (cf. Fig. 1, Pt. I) and rabbits), and also in animals whose brain stem was divided at level 1; the results obtained appear in Tables I and II and are illustrated in Figs. 1, 2 and 3. In some cases continuous ventilation was used but generally the animal breathed the excess of CO_2 or lack of O_2 through valves.

The effect of breathing an excess of CO_2 shows itself in the intact animal (cat) by a sequence of events which is very characteristic and constant (Fig. 1). First for ½–1 minute, the inspiratory tracing progressively increases in height (apneustic centre stimulated); at the end of this period the expiration deepens and becomes active (expiratory centre stimulated). The rate of breathing now begins to increase continuously (vagus and pneumotaxic centre stimulated), until the limit of maximal response is reached in 2 or 3 minutes. Provided that a sufficiency of O_2 is supplied, respiration continues at this level for a long time without apparent harm, even when large amounts (over 20 p.c.) of CO_2 are employed. Thus in a typical experiment, a rabbit (normal respiration 65 p.m.; pulse rate 260 p.m.; blood-pressure 140–150 mm.) breathed 30 p.c. CO_2 with 25 p.c. O_2 for two hours, at the end of which time there was hyperpnœa of normal type (resp. 48 p.m.) the heart-beat was slower (120 p.m.) but stronger, and the blood-pressure was steady and high (150 mm.). The excessive amount of CO_2 maintained a moderate anæsthesia without continued administration of ether. The CO_2 was now

increased to 60 p.c. and the O_2 to 40 p.c. Anæsthesia became very deep, the respiration very slow (12 p.m.) and the heart-beats, though regular

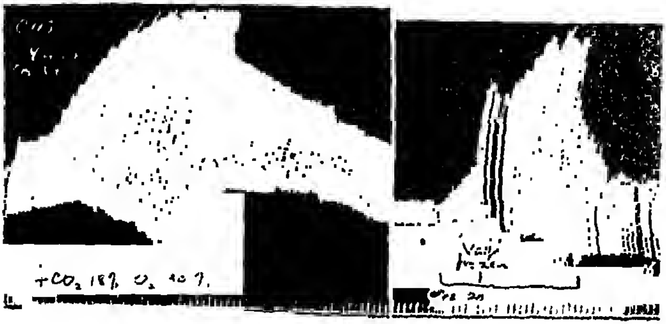

Fig. 1. Fig. 2.

Figs. 1 and 2. Cat. Thoracic tracing. Fig. 1. Effect of breathing 18 p.c. CO_2. Vagi intact. *Time tracing 5 secs. Insp. up. Fig. 2. Vagi frozen during inhalation of 20 p.c. CO_2.*

and very powerful, were infrequent (61 p.m.). After ten minutes the blood-pressure fell progressively to 40 mm. The 30 p.c. CO_2 mixture was then re-administered and recovery was rapid and complete. The vagi were now frozen, causing slowing (15 p.m.) and deepening of breathing, but the heart-beat and blood-pressure (now 100 mm.) remained satisfactory for over an hour. Breathing the 60 p.c. CO_2 mixture now evoked short apneuses, with prolonged (20 sec.) expiratory phases between the apneuses. The blood-pressure fell gradually, but when after ten minutes the 30 p.c. CO_2 mixture was re-supplied, recovery again took place, the blood-pressure rising again to 100 mm.

Similar results were obtained in cats, so that we may conclude that respiration of CO_2 does not prove rapidly detrimental till its concentration is over 30 p.c. When yet higher percentages are inspired they probably prove harmful by paralysing the central nervous system from above downwards, like overdosage with any other anæsthetic. Death in asphyxia must therefore result solely from lack of oxygen. When after a short inhalation of CO_2 (2–15 minutes) air is re-admitted freely, the inspiratory and expiratory increases rapidly lessen concurrently, but for a minute or two the rate of breathing may continue to increase as it gets shallower (Fig. 1).

In ten cats breathing from 5 to 20 p.c. of CO_2 the rate of breathing

increased from an average of 24 to an average of 36 per min., *i.e.* a 50 p.c. increase. The rate soon after the re-admission of pure air was 25·6 p.m., the height of the tracing (by no means a reliable index of the amplitude of the breathing) increased to between two and three times its original amount.

In seven experiments the vagi were divided during CO_2 dyspnœa (Fig. 2). Table I shows the results.

TABLE I. Effects of vagotomy during respiration of excess of CO_2 (Cat). The figures give the respirations per min.

	Exp.	Rate on air	CO_2 %	O_2 %	Rate on CO_2 before vagotomy	Rate after vagotomy on CO_2 at first	Later	Rate after vagotomy on air
Brain stem divided at level 1	1	40	5½	22	30	16	28	18
	2	48	7	30	60	12	12	12*
	3	18	13	27	24	4	4	4†
	4	18	16	50	28	18	22	24
Brain stem intact	5	18	10	30	18	11	12	13
	6	33	10	30	42	21	27	15
	7	28	20	30	30	14	26	16

* Paraffin injection. † Pneumotaxic centre damaged.

The average rate before inhaling CO_2 was 29, at the moment before vagotomy the rate was 36, for half a minute, thereafter it was 13·7; this rate then increased again to 18·7; when air breathing had been resumed for some minutes the rate fell again to 14·6 per min.

In a series of ten cats breathing various percentages of CO_2 after vagotomy had been performed the average figures before, during and after CO_2 inhalation were 13·8, 19·7, 15·1 respectively, the proportionate increase during the experiment was 42·8 p.c., so that the respiratory rate increased nearly in the same ratio after vagotomy as before, though the actual rates were all much slower. The individual figures in this series are shown in Table II and Fig. 3.

TABLE II. After vagotomy effects of breathing excess of CO_2 (Cat).

Exp.	Brain stem	CO_2 %	O_2 %	Resp. per min. on air	Resp. on CO_2	Resp. after on air
8	Intact	5	16	10	24	15
1	Level 1	5½	22	18	26	20
9	—	10	30	12	14	14
5	Intact	10	35	12	14	12
6	Level 1	10	30	18	24	20
10	Intact	12½	27	17	28	22
11	Level 1	12½	30	8	10	9
12	—	16	50	12	13	8
13	Intact	18	30	15	20	15
7	Intact	20	30	16	24	16

Exps. 1, 5, 6, 9, were on cats referred to in Table I.

From these experiments it is plain that using percentages of from 5 to 20 p.c. of CO_2 every animal showed a distinct power to increase the

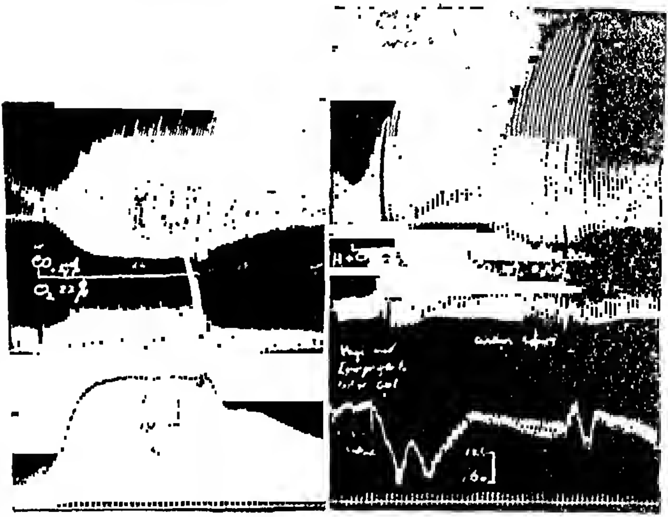

Fig. 3. Fig. 4.

Figs. 3 and 4. Cat. Thoracic, abdominal and blood-pressure tracings. Vagi cut. Fig. 3. Decerebrate inhalation of 5·5 p.c. CO_2. Fig. 4. Inhalation of 2·5 p.c. O_2 and later of 10 p.c. CO_2.

rate of breathing as well as the depth, except Exps. 2 and 3 in Table I, and in each of these the pneumotaxic centre was known to be seriously damaged. There seems then no doubt that the pneumotaxic centre when healthy can, without the aid of the vagus, increase the rate of respiration. As explained above (while treating of the effect of vagal influences during apneustic respiration) the vagus constantly diminishes both the height of inspiration and the depth of expiration and thus tonically quickens the breathing and renders it shallower, so that vagotomy slows and deepens respiration at its apneustic sources whether the chemical calls for hyperpnœa are great or small. The finer vagal adjustments are, however, probably effected through the pneumotaxic centre.

In almost all my experiments the increase of rate after vagotomy took over a minute to appear, and often two minutes to declare itself

fully. The same is not infrequently the case when the vagi are intact. Since in his article (p. 307) Scott states that "although the animals breathed these mixtures usually for less than a minute the rhythm is given in so many per minute," it is not surprising that he usually found no increase of rate after vagotomy. Scott's animals were all chloralised (p. 304); this is not nearly so satisfactory as decerebration since chloral depresses the excitability of the pneumotaxic centre. Thus any increase of rate was apt to be vagal, and to cease after vagotomy. He also omits to mention the rate of respiration after the experiment. The above points explain the difference between Scott's negative results and my positive results.

The effect of breathing low percentages of O_2 was investigated. It was found that, as stated by Haldane and others, very little change was seen in the respiration till the O_2 became less than 12 or even 10 p.c. When an animal breathed 8 p.c. O_2 before vagotomy the rate increased from 20 to 23 per min. After vagotomy the figures were 16 and 19 respectively. There is a much greater increase in the amplitude of the breathing after vagotomy, which corresponds with the slower rate. The increase is entirely inspiratory and as shown in my preceding paper is due to stimulation of the apneustic centre.

Vagotomy performed during respiration of H_2 pure or of $2\frac{1}{2}$ p.c. of O_2, increases the height of the inspiratory tracing enormously; the rate remains almost unaffected. Active expiration does not occur. The purely inspiratory effect of O_2 lack after vagotomy is particularly well seen in Fig. 4, and the difference of the dyspnœa due to O_2 lack and that resulting from excess of CO_2 is apparent.

After vagotomy O_2 lack is as one would expect even more rapidly fatal than usual. In a cat in which after vagotomy, CO_2 excess did not increase the rate of breathing there was marked quickening of the respiration when O_2 was concurrently lacking, and in this experiment it appeared that the increase of rate coincided with diminution in depth of breathing, an evidence of apneustic failure with a still effective pneumotaxic centre.

The Source of Vagal Respiratory Impulses.

A large number of experiments (over 100) were made on cats and rabbits with a view to determining the origin of the vagal impulses affecting the respiratory centres. The methods of investigation employed were chiefly closure of the trachea at every phase between full inspiration and full expiration, both before and after vagotomy, and during powerful positive and negative ventilation.

Closure of the trachea.

Vagi intact (Fig. 5). (*a*) If the closure is effected at the height of inspiration (INSP. V.I.) attempts to empty the chest so as to regain the zero position of pressure and distention (*i.e.* expiratory efforts) predominate. Since the chest was full when closed, lack of oxygen does not play much part in the immediate effects observed.

Fig. 5. Cats. Closure of the trachea in different conditions of the lungs (see text). V.I., vagi intact; V.C., vagi cut; +, positive pressure; –, negative pressure.

(*b*) Closure at the moment when the chest has been emptied by expiration (EXP. V.I.) produces more complicated effects. First the respiration becomes slow and the inspiratory rises on the tracing have rounded apices. After half a minute or so, each inspiration ends in a gasp. Soon the apneustic part of the inspiration lessens, the superimposed gasp increasing *pari passu*. After a minute only gasps interspersed with expiratory spasms occur. These effects are obviously in part due to lack of oxygen causing, as usual, failure of the respiratory centres in order from above downwards; the primary inspiratory phase being the only one attributable to vagal impulses of pressure and position.

(*c*) Closure midway between inspiration and expiration (MID. V.I.) gives effects very much the same whether the vagi are cut or not; since neither deflation nor distention was present to arouse any continuous vagal impulses of position or pressure and O_2 lack is not at once serious, respiratory movements simply continue slowly and deeply as if the vagi had been cut.

Vagi cut (Fig. 5, INSP. V.C. and EXP. V.C.). Closure in any of the above positions after vagotomy gives in all cases very much the same result. The reason of this appears to be that the centres (vagal impulses having been eliminated by tracheal closure) had already accommodated themselves to breathing without vagal assistance. Closure therefore acts like making an animal, whose vagi had previously been cut, breathe an atmosphere lacking in O_2 and overladen with CO_2. As pointed out above vagotomy performed during CO_2 dyspnœa causes for a short time much disorganisation of the breathing but after a minute or so the centres accommodate themselves and make a very fair effort to deal with the excessive CO_2 by increasing both the height and rate of respiration. If vagotomy had been performed some time before inhalation of CO_2 is begun there is no disorganisation, compensatory hyperpnœa of central origin appears at once in an ordered and regular manner. Similarly, closure of the trachea some time after vagotomy, merely evokes a centrally controlled hyperpnœa with no special bias towards either inspiration or expiration, since the brain stem is no longer informed of any abnormality of either position or pressure.

Excessive distention and deflation (Fig. 5). Distention being associated with positive pressure is much more effective than deflation and negative pressure. Closure of the trachea during distention at a pressure of 30 to 50 mm. Hg (++ V.I.) causes complete inhibition of the normal inspiratory tonus with expiratory apnœa. Sooner or later this is broken by typical gasps released by the inhibition of the higher centres and by the asphyxial state of the blood. If less pressure is employed (+ V.I.) the inhibition lasts only momentarily and inspirations of normal type are then resumed. If during the apnœa which results from excessive distention of the lungs the vagi are cut (++ vagi cut), rounded apneustic inspirations immediately occur (the inhibition which must have been vagal having been removed); these are quite distinct from the gasps just mentioned. It is therefore clear that intense distention is during its continuance an adequate vagal stimulus but it must be borne in mind that here we are dealing with pressures such as can never occur during natural inspiration and which are distinctly nocuous, for emphysematous changes can be seen post mortem.

Closure of the trachea during suction (− V.I.) gives rise during the first few seconds to inspiratory effects of apneustic type and then attempts at respiration are resumed with a gasp superimposed on each inspiration, the effects noted under closure in expiration being reproduced with some exaggeration. After vagotomy closure in deflation and also

closure in distention show no special effects of nervous origin, the results differing only mechanically from closure after vagotomy in any other position.

The immediate effects of vagotomy during closure of the trachea.

Vagotomy in this condition never slows the respiration which as above explained had already assumed the centrally determined rhythm. This indicates that the accelerating effects of the vagal impulses depend not on the amount of distention or deflation but upon the passage of air outwards and inwards; the effects which are observed are all in the direction of a diminution of efforts which would, but for the closure, result in resumption of the zero point of position and pressure, or rather in and out play of inspiration and expiration round that point. Vagotomy during closure in full inspiration thus diminishes the expiratory efforts, which were occurring. Vagotomy during closure with the chest in the expiratory position has little effect except that it increases the gasps which were superimposed on the attempted inspirations. Vagotomy during closure midway has no appreciable effect (Fig. 5, MID. V.I. V.C.). Vagotomy during great distention has been referred to above.

Rhythmic ventilation.

As stated by Hering and Breuer and confirmed by Head, rhythmically repeated ventilations dictate the rhythm of breathing as long as the vagi are intact. After vagotomy this does not happen. This I consider to be due to vagal impulses aroused by the currents of air as they pass in and out through the trachea and bronchi. It is of interest here to note Jappelli's(5) observation that in man, dogs, and rabbits, rhythmical stimulation of various other nerves, *e.g.* sciatic, could in like manner prescribe the rate at which respiration occurred.

That such air currents are sufficient to stimulate the vagus can be proved, after low vagotomy, by blowing air inwards through the larynx and upper part of the trachea, while continuous ventilation is performed through a tracheotomy opening below the portion of trachea blown into (Fig. 6). The downward current of air inhibits inspiration or apneusis quite as efficiently as electrical stimulation of the vagus. Similarly by blowing air upwards expiration may be inhibited, but this effect is less striking and less easily demonstrated.

Again, during continuous ventilation of the lungs performed so that only midway distention is caused we find that active expiration tends

Breuer, Head and others to prove that distention and deflation of the lungs and not changes of pressure were the causes of the vagal impulses, favour with equal cogency, and none of them refute, the view that the in and out currents of air are the normal vagal stimuli.

5. Loewy's (8) experiment also supports the air current theory. He opened the right pleura of a rabbit after the animal had been breathing O_2 for some time. The O_2 was soon absorbed and the lung collapsed completely. Yet the respiration continued normally at 66 per min. Loewy now cut the left vagus. The respiration immediately assumed the complete vagotomy type at a rate of 34 per min. He then performed artificial respiration and the respiration assumed the pump rate, or a multiple thereof. This experiment, which I have repeated in rabbits and cats, strongly supports the air current view of vagal excitation, for on the distention and collapse theory the collapsed right lung should have produced, especially after cutting the left vagus, not the normal vagotomy effect but a marked inspiratory excitation. Loewy's experiment falls into line with my observation that closure of the trachea in any natural position between full inspiration and expiration acts like vagotomy and that very little effect results if the vagi are frozen during such closure.

6. During continuous ventilation, when gas is pouring downwards constantly through the air passages it evokes persistent expiratory efforts even if there is no marked distention of the lungs and though O_2 40 p.c. N_2 60 p.c. is used. Vagotomy abolishes this effect, and it ceases at once if the current is stopped and the lungs are allowed to collapse, in which case the air passes for a time outwards and inspiratory efforts occur.

Now, with regard to the variable effects which follow closure of the trachea, these show themselves most markedly when closure is effected after powerful distention or deflation, and that they do result from vagal influences is proved by the fact that they are not seen after vagotomy. Thus, as pointed out above, if during the expiratory apnœa which results from closure of the trachea after violent distention of the lungs, the vagi are cut, the apnœa ends at once and attempts at inspiration and expiration are resumed forthwith. This experiment alone is sufficient to indicate that the accelerating impulses are not the only ones carried by the vagi from the lungs. These nerves must also convey postural impulses relating to the position of the lungs in regard to distention and deflation and to the intra-pulmonary air pressure. We may suppose that at least one use of these impressions is to protect the lungs against either excessive distention or pressure; hence it seems natural that the response called

forth, say, by distention, is such as would resist further distention, *i.e.* an expiratory effort. In any case it is a fact that the response to any vagal message of pressure or position is always in the direction of safety and conservation of energy and muscular effort, viz. towards attaining the atmospheric pressure and the zero position midway between distention and deflation. In accordance with these views, we find that vagotomy performed during closure with the chest midway between inspiration and expiration (*i.e.* zero position) has no effect (Fig. 5). Respiration simply continues at the central rhythm already induced by the closure of the trachea. If, however, distention or deflation is present, efforts to attain the zero position are made as indicated above (Fig. 5).

On the whole I think it fair to conclude that in easy breathing the air currents as they pass over the ciliated bronchial mucous membrane form the natural vagal stimulus. But when distention or deflation assume abnormal degrees, they do give rise to postural vagal impulses which are not, however, concerned primarily with acceleration of breathing but with the tendency to attain the zero position of greatest ease and least exertion.

The vagi must also carry to the brain impulses aroused by painful and other nocuous stimuli, and these may have definite respiratory effects. Thus inhalation of an irritant gas gives rise (in both cats and rabbits) to active expiration, an effect which ceases after vagotomy.

I have observed a number of occurrences which suggest that deep sensations from the abdominal and thoracic parietes and viscera are of considerable respiratory importance. These impulses reach the cerebrum partly by the vagi and partly by the dorsal nerves, and among other facts they may explain the cessation of diaphragmatic respiration during acute abdominal inflammation or peritonitis. Another gastric vagal effect is hiccough.

Subjectively, we are aware of a number of these impulses, notably of the passage of air inwards and outwards, of pain, and of the position of the thoracic and abdominal walls and they probably therefore have important cortical respiratory effects. Since, however, the cerebrum and cerebellum can both be removed in their entirety without alteration in the rhythm of unconscious respiration, we must assume that the respiratory centres are independently capable of regulating the breathing in response to the pulmonary and bodily impulses just mentioned, and to the condition of the blood.

The trigemini have no tonic action on respiration for they can be cut without disordering it. If these nerves are irritated they have a protective

effect in one or other of two ways: they may, even after decerebration, aid in the expulsion of the noxious substance by giving rise to sneezing, while if an irritant gas is inspired through the nose temporary apnœa results; in the case of the tortoise this apnœa may last two hours or more. That after section of the 5th nerve this reflex ceases was, I believe, first pointed out by Kratschmer. The probable arrangement of the centres and their connections are diagrammatically represented in Fig. 7.

Two cases have recently been observed in which the apneustic type of respiration occurred in man. The first is recorded by Kirkwood and Myers(9). In this case I found hæmorrhages in the brain stem just above the striæ acousticæ, similar to those caused in cats by dragging on the auditory nerves during attempts to cut them. The brain stem of the second case is about to be examined.

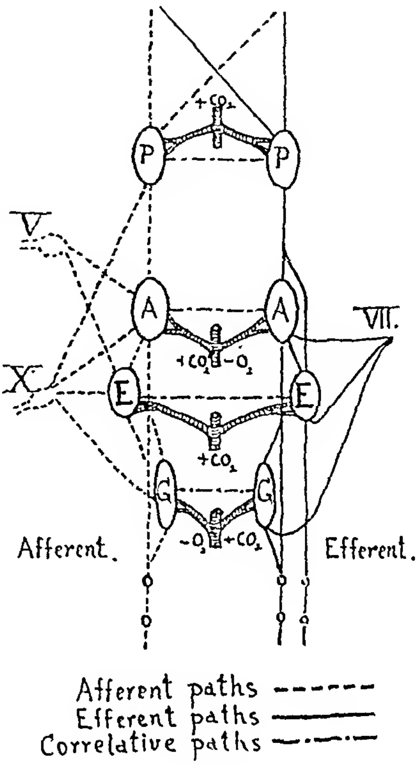

Fig. 7.

Remarks and Conclusions.

During normal respiration neither inspiration nor expiration attains its full extent since the vagal impulses are constantly limiting alternately apneusis and expiration, especially the former. Since the vagus thus tonically accelerates the respiration in all cases, whatever its depth and rate may be, vagotomy always slows and deepens the breathing.

The H-ions in the blood continuously increase the excitability of the pneumotaxic centre so that it periodically discharges and inhibits the tonic apneusis and thus in conjunction with the vagus determines the rhythm of respiration of normal type.

Apart from vagal influences, *e.g.* after vagotomy, the height and depth of unconscious breathing is determined primarily by the stimulating effect which CO_2 excess exerts upon the apneustic, and when the excess is pronounced, upon the expiratory centres.

During waking life cerebral influences modify respiration to a variable degree, often profoundly. There is now no fixed base line (position of complete relaxation) to which each expiration returns, such as is seen during sleep and anæsthesia. The lower edge of a respiratory tracing taken during consciousness varies up and down continually.

When CO_2 is present in moderately increased amount the first effect is that the inspiration becomes deeper from stimulation of the apneustic centre, if the increase of CO_2 continues, expiration is also exaggerated, from stimulation of the expiratory centre. If these efforts do not succeed in diminishing the excess of CO_2 the pneumotaxic centre by becoming more excitable increases the rate of respiration and the larger currents of air passing through the bronchi stimulate the vagal endings more powerfully than usual, thus further adding to the acceleration of breathing by limiting more drastically than usual the continuance of both inspiratory and expiratory movements.

If during dyspnœa due to CO_2 excess the vagi are cut, the slowing and deepening of the breathing is much more marked and expiration becomes more intense and spastic than if vagotomy is performed during quiet respiration; an evidence that the vagi were carrying inhibitory messages more powerful than usual (Fig. 2).

Even after vagotomy the respiration may quicken very markedly during CO_2 dyspnœa, it is therefore clear that the vagus is not the only accelerating mechanism. *It* appears *that* the pneumotaxic, apneustic and expiratory centres are all rendered more excitable by excess of H-ions in the blood circulating through them and hence respond more actively than usual, thus quickening the breathing (Fig. 3).

When the pneumotaxic centre is damaged or is failing from any cause, the response to CO_2 excess is much less brisk than normal and if now the vagi are cut the respiration becomes enormously increased in height and slowed almost to the point of apneusis and thereafter no increase of rate occurs. This shows that it is largely through the pneumotaxic centre that the CO_2 central acceleration is effected.

The effects of oxygen lack on respiration vary with its intensity. A moderate lack of O_2 down to 12 p.c. produces hardly any dyspnœa at all, and the slight effect it ultimately shows is possibly due to increased sensitivity to CO_2. Greater lack of O_2 (2–8 p.c.) stimulates apneusis, thus heightening inspiration for a short time, but soon it paralyses first the pneumotaxic centre and thus apneuses appear. The centres at the striæ region next fail, gasping resulting, and soon this also stops and death ensues.

Asphyxial death is entirely due to O_2 lack. Very large amounts (20 to 30 p.c.) of CO_2 can be breathed for several hours on end without immediate danger to life.

It is probable that various nerves of deep sensibility supplying the thoracic and abdominal parietes and contents also aid in regulating respiration, and on occasion the trigemini certainly do, *e.g.* by inducing sneezing and by inhibiting respiration altogether when an irritant gas enters the nostrils.

The accelerating (inhibitory) vagal effects are normally evoked by the inrushing and outflowing currents of air; the former inhibits the inspiration which caused it and conversely the latter inhibits expiration.

Besides these accelerating impulses the vagi also convey to the brain stem postural impulses relating to the condition of the lungs as regards distention, deflation, positive and negative pressure, etc. The effect of these impulses is to evoke such muscular efforts as will make for quiescence *at the zero point of pressure and the mid-way position of repose between* inspiration and expiration. Within the pressures and limits of ordinary easy breathing these postural and pressure impulses are unimportant.

I have again to thank Prof. C. J. Martin for putting his laboratory at my disposal. I am also much indebted to him, to Prof. J. N. Langley and to Prof. E. H. Starling for their helpful interest in my investigations.

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THE ANAEROBIC PROCESSES INVOLVED IN MUSCULAR ACTIVITY. By W. HARTREE[1] AND A. V. HILL.

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EXPERIMENTS on isolated muscle have shown that considerable quantities of lactic acid are produced rapidly during stimulation, and experiments on man have emphasised how extremely sudden this production of acid may be. An athlete may, in ten seconds of violent effort, produce an oxygen "debt" of about four litres(2), an amount corresponding to the oxidative removal of about 30 gms. of lactic acid, or of 0·1 p.c. in the active muscles. During ten seconds the circulation, starting from its resting rate, cannot possibly be able to play any appreciable part in neutralising this acid or in removing the acid or the CO_2 which it has replaced. The acid liberated must necessarily be dealt with at the moment solely by the muscle fibres themselves. Immediately after exercise there is a rapid rise in the respiratory quotient and a considerable elimination of the CO_2 turned out by the lactic acid from bicarbonate in muscles and blood. Later on there is a corresponding retention of CO_2 as the lactic acid is removed in recovery. During the few seconds, however, of the exercise, this CO_2, together with the lactic acid, must necessarily remain inside the muscle fibre itself. It is of interest to inquire how this amount of lactic acid is accommodated within the muscle in addition to the CO_2 already there, without a rise of hydrogen ion concentration likely to destroy its colloidal structure. The same question arises from a consideration of the stimulation of frog's muscle. A short tetanic stimulus, far too short to allow a noticeable amount of CO_2 to escape, can produce a considerable amount of lactic acid in the muscle.

It is obvious that the lactic acid must be neutralised. It is a relatively strong acid, being 10 p.c. ionised at the maximum concentration at which it can occur in the body. A fatigued muscle certainly has not the hydrogen ion concentration which it would have were lactic acid free within it(6). Meyerhof(4), in a recent paper, has emphasised that in a frog's muscle the absolute amount of bicarbonate present, as determined from the CO_2 driven out from the muscle by excess of acid, is quite

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inadequate in amount to neutralise the acid liberated in severe stimulation. Even if all the CO_2 were driven out by acid, there would only be about 1/7 to 1/10 as much bicarbonate as would be required. A similar argument applies to phosphates. According to Laquer (3) there is about 0·3 p.c. of inorganic phosphate in muscle, namely a mixture of KH_2PO_4 and K_2HPO_4. Assuming with Meyerhof that the reaction of fatigued muscle cannot change more than from a pH of 7·5 to one of 6·9, this would correspond, according to Michaelis, to a change from a mixture of 8·6 c.c. of Na_2HPO_4 + 1·4 c.c. of NaH_2PO_4 to a mixture of 6·2 c.c. of Na_2HPO_4 + 3·8 c.c. of NaH_2PO_4. Hence, assuming that there is 0·3 p.c. of H_3PO_4 present in muscle, there would be only about enough phosphate available between these reactions to neutralise about one-quarter of the lactic acid known to be liberated. If all the bicarbonate and all the phosphate available between these reactions were used up, there would be insufficient to neutralise one-half of the lactic acid formed.

It is obvious, therefore, that the alkaline salts present in muscle are not sufficient in amount to neutralise the acid liberated in a brief violent effort, and still less in a prolonged violent effort. The answer, suggested by Meyerhof, to the problem is that the muscle possesses in its colloids a far more effective buffer than phosphate or bicarbonate, and has it in much larger amounts. This hypothesis of Meyerhof, by providing a much greater heat of neutralisation, fits in better with what is known of the heat liberated during contraction and relaxation. Its necessity can be demonstrated in another way. If it be assumed that bicarbonate is in the same concentration in muscle as in blood (it can hardly be higher) and that the CO_2 in muscle is at the alveolar pressure of CO_2, then when 0·1 p.c. of lactic acid is suddenly liberated and neutralised by bicarbonate and the CO_2 so formed is unable momentarily to escape, it can be shown by a simple calculation (employing Henderson's equation) that the hydrogen ion concentration would rise some 18 times. No such change, however, has been detected in isolated muscle (6), and it is difficult to imagine that such a change of cH in the intact animal could be tolerated without disaster. If we imagine 0·15 p.c. of lactic acid to be set free, a similar calculation would require the cH of a man's muscle to be increased about 45 times by about 15 seconds of exercise. In the case of complete exhaustion the change would be enormous. Similarly, if we consider the case of a phosphate mixture the change in cH necessary to allow the neutralisation of the comparatively large amounts of lactic acid formed in exercise would also be enormous.

It is obvious, therefore, that in muscle there must be some more

effective means for neutralising acid suddenly set free; in blood the existence of such means can readily be demonstrated.

Numerous observations have been made of the effect on the cH of blood of adding acid to it. None, however, to our knowledge, have been recorded in which the conditions were exactly analogous to those now being discussed, namely, in which the CO_2 turned out by the added acid is unable momentarily to escape. Experiments therefore have been made on our behalf by Miss D. M. Thomas, in which defibrinated fluorided sheep's blood, carefully brought into equilibrium with air containing CO_2 at a pressure of about 40 mm., had various quantities added to it of lactic acid (standardised by titration), the cH being then determined by the Dale-Evans method of dialysis. A large quantity of blood was equilibrated, 10 c.c. was pipetted off into six test-tubes, a few drops of oil were added at once to prevent contact with the air and escape of the CO_2, and then small quantities of a 7·75 p.c. solution of lactic acid were added from a capillary pipette. The blood in each test-tube was carefully stirred to ensure mixing of the acid without escape of CO_2, and 2 c.c. withdrawn and its cH measured in the usual way. The mean results of three complete consistent experiments on different samples of blood are given in Fig. 1. We see that the addition of ·05 p.c. of acid raises the

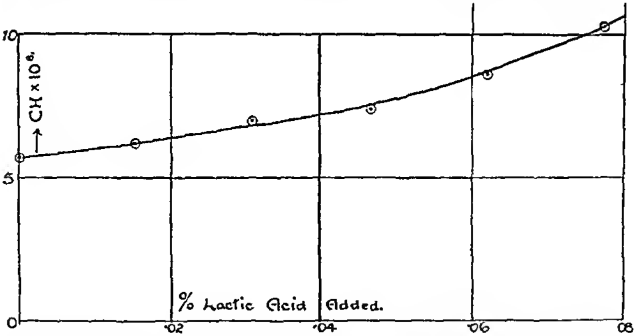

Fig. 1. Change of *c*H on adding lactic acid in known final concentration to defibrinated sheep's blood without escape of CO_2. The blood had previously been equilibrated with air containing CO_2 at 41 mm. pressure.

*c*H only 1·35 times, while the addition of 0·1 p.c. of acid raises the *c*H under similar conditions only 2·2 times (result slightly extrapolated). Were blood simply a bicarbonate solution the *c*H would have risen not

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2·2 times but 18 times. There must be another and more effective buffer than bicarbonate in the blood, namely, as is commonly assumed, a sodium or potassium salt of hæmoglobin, and (to a much less extent, Milroy (5)) of the protein of the plasma.

It would seem probable therefore that there is, in muscle also, some alkaline protein salt analogous to the sodium or potassium salt of hæmoglobin in blood, capable like hæmoglobin of releasing the base to neutralise acid suddenly appearing in it, and causing the change of *c*H to be only very small, owing to the weakness of the protein acid so produced. It will be a matter of great interest to attempt to extract from living muscle some alkaline protein buffer possessing this extreme power of maintaining the constancy of the *c*H.

In a following paper in this Journal W. K. Slater shows that the whole of the heat liberated in an anaerobic contraction (according to Meyerhof 370 calories per 1 gm. of lactic acid produced) can be explained as due to the formation of lactic acid from glycogen and its subsequent neutralisation. The only assumptions necessary are (*a*) that in the experiments in which the 370 calories per 1 gm. of lactic acid were determined, about half of the lactic acid was neutralised by bicarbonate or phosphate and half by alkaline protein buffers, and (*b*) that at the end of the partial cycle of the initial breakdown the muscle is unchanged except in so far as glycogen has become sodium or potassium lactate, any intermediate products, if they exist, being in the same concentration finally as they were initially. It would seem almost certain therefore that the anaerobic contraction regarded as a whole is accompanied simply by the glycogen-lactic acid breakdown, or at any rate not by any other chemical processes involving appreciable exchanges of energy. To fill in the details of the picture, however, is more difficult. There are presumably various intermediate breakdowns which must be reversed or compensated before the partial cycle is complete. For example, the immediate cause of contraction may be the formation of lactic and phosphoric acids from "lactacidogen" (Embden) which is then restored from glycogen and phosphoric acid at some time unknown but before the cycle is complete. The heats of these reactions are not determinable at present. Moreover, during the mechanical response mechanical potential energy is liberated, and during relaxation this mechanical energy is presumably dissipated as heat. Heat is known to be liberated during relaxation in amount (in the case of the isometric twitch) about 40 p.c. of the total initial heat. This cannot however be attributed simply to degraded mechanical energy since we do not know

what exothermic or endothermic reactions may accompany relaxation. Without more knowledge, therefore, of the physical and chemical events it is not possible yet to utilise further the outlines provided by the thermodynamical method. Since, however, the chemical details, when we know them, must certainly fit into the thermodynamical picture, we have endeavoured to establish the latter as firmly as possible, as will now be further discussed.

The only point on which we felt any serious doubt of the general accuracy of the results given in our previous papers on the distribution of the heat between the several phases of muscular contraction, was in respect of the delayed anaerobic heat (1). The experiments seemed conclusive enough in themselves, but the result was so unexpected and so difficult to interpret that we have continually wondered whether it was not due to some systematic error. During the last nine months, therefore, we have attempted, but completely without success, by any and every means to eliminate this delayed heat production, or to explain it on purely physical grounds. We have concluded that there can be no doubt of its existence, and its general properties are as described below. Its origin is more fully discussed by W. K. Slater in a following paper.

Following a short tetanic stimulus of an isolated muscle, at 20° C. in nitrogen, there is an evolution of heat lasting for some minutes (10 to 15 at least) after the contraction has passed off. Further experiments have been made of the same kind as the previous ones but especially (*a*) with longer times of stimulus, (*b*) at lower temperatures, and (*c*) with specially purified nitrogen. Besides the ordinary precautions required in such experiments it was now necessary: firstly to have a very steady zero for the photographic records, both on account of the smallness of the quantities to be observed, and also because the records must be continued for at least eight minutes; secondly, to consider the possible effects of traces of oxygen left in the chamber; and thirdly, to consider the possible inaccuracy on physical grounds of the method of analysis employed.

Most of the experiments were carried out using the same method as before. In each case the muscles were first left in the thermopile chamber for an hour, in Ringer's solution which had been previously boiled. This was then blown out by nitrogen from a cylinder, bubbled in very minute bubbles through a solution of alkaline pryogallol. It was later found on analysis that such nitrogen might still contain as much as $1\frac{1}{2}$ p.c. of oxygen as impurity. The amount dissolved in the muscle at this pressure might conceivably be sufficient to account for a considerable part of the

delayed heat after a short stimulus, but it could not account for more than a small part of it after a long stimulus.

In our previous paper on this subject the average of the delayed heat at 20° C. for a short tetanus (up to ½-sec.) was found to be 0·5 times the total initial heat. We have obtained the following results in our recent experiments.

(*a*) *Absolute value.* In five experiments at 20° C. with times of tetanus from 1 to 10 seconds, the corresponding value was between 0·36 and 0·23 for 12 observations, with a mean value of 0·30.

(*b*) *Effect of temperature.* About ten reliable experiments were carried out at 0° C., the time of stimulation varying from a single shock to one second. In 12 observations the corresponding value was between 0·55 and 0·22, with a mean value of 0·34, showing that there is very little effect of temperature on the absolute size of the delayed heat.

(*c*) *Completeness of absence of oxygen.* In the last six experiments at room temperature (about 12° C.) nitrogen from a cylinder was passed through a hydrosulphite solution (10 gms. $Na_2S_2O_4$, 5 gms. NaOH, to 100 c.c. of water) and probably contained less than ·05 p.c. of oxygen. In eight observations the corresponding value lay between 0·37 and 0·24, with a mean value of 0·29. This last number is practically the same as the mean found in (*a*), in the presence of less pure nitrogen: it seems definitely to exclude the possibility of the phenomenon discussed being due to the presence of remaining traces of oxygen.

Although the total amount of the delayed anaerobic heat does not appear to be affected much by temperature, or by duration of stimulus, there are noticeable differences in the time curves of the heat production. It should be remarked, however, that the actual curves given in our previous paper for the rate of delayed heat production are undoubtedly contaminated to a small degree by the presence of oxygen as an impurity in the nitrogen. The principal differences now observed may be summarised as follows:

(*a*) At higher temperatures (up to 20° C.) a short stimulus (1/20 sec. or less) gives rise to a delayed heat production whose rate is zero or very small soon after the stimulus, but increases to a maximum at about 2½ minutes after the stimulus; whereas for long stimuli (1/2 sec. or more) the rate of delayed heat production falls rapidly from the comparatively large value which occurs very soon after the stimulus.

(*b*) At low temperatures (0° C.) the difference of the initial form of the heat rate curve due to alteration of stimulus from a single shock up to a tetanus of one second was not appreciable; in some experiments the

rate fell rapidly from the comparatively large initial value occurring very soon after the stimulus, while in other experiments the rate increased from a very small value soon after the stimulus to a maximum occurring after about $2\frac{1}{2}$ minutes. Now it is observed in those cases in which the rate falls from a large initial value that not only is the total delayed heat larger than in other cases, but also there is usually (after the first rapid fall) a distinct rise in the curve, producing the maximum rate at about $2\frac{1}{2}$ minutes after the stimulus, as shown in the figure.

From a consideration of these facts it would seem likely that the effects observed are the resultant of those arising from two separate heat productions, the first of which depends on the temperature, or the time of stimulus, or both, while the second is independent of these circumstances.

As regards the first part it is evident that when the delayed heat starts early at a large rate, this early rate cannot be well estimated, since its effect is superimposed on that of the initial heat. The galvanometer deflection is large and altering rapidly. Furthermore it is a matter of speculation how to divide up the curve shown in Fig. 2 into two curves

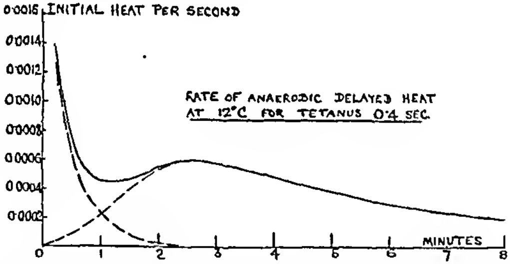

Fig. 2. Course of the delayed anaerobic heat production in muscle, showing the characteristic secondary rise in the curve when the time of stimulus is not very short. The dotted curves represent two hypothetical simple curves making up the more complex actual one. In many cases the curve is similar to the second dotted one. The second curve provides nearly the whole of the delayed anaerobic heat.

similar to those shown dotted in the figure. Thus it is better to estimate the total of the second part, only for those cases in which the first part is very small or negligible, namely, for short stimuli at high temperatures, or for those cases at low temperatures in which the delayed heat rate rises from a small value. The mean value of the total delayed heat calculated in this way for a short tetanus, in four experiments at room

temperature (12° C.) with the specially purified nitrogen, was 0·25, and in five experiments at 0° C. with the nitrogen first used was 0·28. Even in these cases a possible small allowance should perhaps be made for the small amount of heat occurring in the first part, but this is certainly less than 10 p.c. of the total. The explanation of the first part is not clear. It seems to depend upon the temperature, and it is conceivable, though not likely, that it is due to the oxygen still remaining dissolved in the muscle. It seems certain, however, apart from the possible effects of errors which we will next consider, that there is a definite delayed anaerobic heat production, independent of temperature both in magnitude and in manner of production, and independent of the duration of stimulus, of total amount between 0·2 and 0·25 times the initial heat, having a maximum rate about ·0006 times the initial heat per second, this maximum occurring about 2·5 minutes after the stimulus.

The one possible source of error of a purely physical nature, which we have considered, lay in the presence of a small amount of muscle beyond the electrode. When the muscle is alive and stimulated, this part is active and produces heat. When it is dead and the control curve is taken, only that part which lies between the electrodes is heated. Hence in the latter case part of the heat produced between the electrodes will be required to warm up the part of the muscle lying beyond the electrodes and the control curve will fall away from its maximum more quickly than it should. The effect of using such a control curve for the analysis will be to give too great a result in the heat production, and as this error must be independent of temperature, etc., it was particularly important to make sure that the effect was not of the same order of size as the calculated delayed heat.

Two methods were employed to see if the effect of this error were appreciable. Firstly, experiments were carried out in which the length of the muscle projecting beyond the electrodes was varied over a wide range. There did not, however, appear to be any connection between the length projecting and the magnitude of the delayed heat as determined by the analysis. This was the case even when only a very small part of the muscle projected beyond the electrode. Under such circumstances it should be noted that the muscle is very thin near the electrode and a large amount of heat is produced by the electric current in this thin portion. This should give exactly the opposite effect to that produced by a large portion of muscle projecting, but in spite of this the anaerobic delayed heat appeared as usual. Secondly, several experiments were carried out on a dead muscle supplied with an extra electrode to the end

of the part projecting beyond the ordinary electrode. The projecting part only was then separately heated to a certain rise of temperature and the subsequent galvanometer readings were compared with those caused when the part between the ordinary electrodes was heated to the same rise of temperature. In this way it was shown that the maximum error, even when as much as one-quarter of the whole muscle was projecting beyond the electrode, is not more than 0·4 p.c. of the maximum galvanometer deflection in the ordinary control. In this way it was amply proved that the error in question could not account for more than about 1/10th of the delayed heat determined in the usual way, and it must usually be much less since (in most experiments) there is only a small part (about 1/10th of the whole weight of the muscle) projecting beyond the electrode.

It appears therefore, making every allowance for possible errors, that the total heat of the delayed anaerobic process is about 0·25 of the total initial heat. Its rate of evolution, as also its magnitude, appears to be independent of the temperature of the muscle. We have several times confirmed our previous result that the delayed heat in the complete cycle in oxygen is about 1·5 times the initial heat, and we will assume, for an *isometric* twitch, that the heat of relaxation is 0·4 of the total initial heat. The following "balance sheet" represents an estimate, to some degree final so far as present methods go, of the heat evolved in the various phases of the muscular cycle.

Isometric contraction of frog's muscle.

Phase	Relative	Absolute, per gm. of lactic acid
Total anaerobic	1·25	370 (Meyerhof)
Total initial (anaerobic or aerobic)	1·0	296
Delayed anaerobic	0·25	74
Initial (contraction)	0·6	178
Initial (relaxation)	0·4	118
Oxidative delayed	1·5	444
Total oxidative	2·5	740

As Slater shows in a later paper, the initial anaerobic heat can be attributed to simple chemical causes, viz. to the transformation of glycogen into lactic acid and its neutralisation by alkaline salts. In the complete oxidative cycle the muscle is finally as it started, apart from the oxidation of a fraction of the 1 gm. of glycogen $(C_6H_{12}O_6)_n$ from which the lactic acid arose. According to Slater the heat of combustion of dissolved glycogen $(C_6H_{12}O_6)_n$ is 3874 calories per gm., hence

$$x=\frac{740}{3874}=\frac{1}{5\cdot 24},$$

so that with the standard relative values adopted here about 5·24 molecules of lactic acid are removed in the complete cycle for each one oxidised.

It is not possible, with the data at our disposal, to decide that the rate of "removed" to "oxidised" is exactly 5·24 : 1. With a delayed anaerobic heat of 0·2 and a delayed heat in oxygen of 1·7 (relative values) the heat in the complete cycle would come to 852 calories and the ratio to 4·66: all these values lie within the limits of possibility. Or again, with a delayed anaerobic heat of 0·35 and a delayed heat in oxygen of 1·3, the heat in the complete cycle would be 630 calories and the ratio 6·01. Both these values again are possible, so far as the results of our experiments go. We can only conclude definitely, therefore, that the ratio of lactic acid removed to lactic acid (or glycogen) oxidised lies between the values 4·7 and 6, with a most probable value of 5·24.

It is important, for the present, to emphasise this latitude. It is possible that the removal process is some kind of "coupled reaction" in which the ratio of "removed" to "oxidised" is that of some simple integers: in this case 5 : 1 and 6 : 1 are both possible. It is also possible, however, that the recovery process is due, as we have supposed elsewhere(1), to some recovery "mechanism," supplied with "power" by the combustion of carbohydrate, in which case the ratio might be expected to vary with the condition of the muscle. At present it is not possible to decide between these alternatives.

SUMMARY.

1. When slaughter-house blood, brought to a tension of 40 mm. of CO_2 has 0·05 p.c. and 0·1 p.c. of lactic acid added, without escape of CO_2, the cH rises to 1·35 and 2·2 times its original value respectively. In a solution of bicarbonate of similar CO_2 pressure and bicarbonate ion concentration, the cH would rise to 7 and 18 times its original value respectively.

2. In muscle 0·1 p.c. of lactic acid can be produced so rapidly by exercise or by stimulation that no appreciable escape of CO_2 or acid is possible. Hence if the hydrogen ion concentration inside a muscle is not to rise to an excessive degree during exercise, there must be some buffer in it much more effective than a bicarbonate solution. The same argument applies to phosphate. Hence it appears to be necessary to assume, with Meyerhof, that living muscle contains, as blood is known to contain, an alkali-protein salt capable of neutralising acid and forming the neutral salt and the undissociated protein.

3. Experiments are described confirming the existence of the small delayed heat production occurring in the complete absence of oxygen in an excited muscle. The rate of evolution of this heat appears to be unaffected by temperature, and its most probable total value to be about 0·25 of the initial heat.

4. Accepting this value, a new "balance sheet" (final as regards methods at present available) is given of the heat evolved in the different phases of muscular contraction, reckoned in calories per gm. of lactic acid set free.

5. From this balance sheet it is found that in the recovery process something between 1 part in 4·7 and 1 part in 6 of the lactic acid removed is oxidised, the remainder being retained as glycogen.

We are much indebted to Miss D. M. Thomas for making the experiments on the hydrogen ion concentration of blood.

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OBSERVATIONS ON THE TAKING UP OF CARBON MONOXIDE BY THE HÆMOGLOBIN IN THE SPLEEN.

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THE object which prompted the experiments described in the following paper was to ascertain the extent to which carbon monoxide administered by the lung penetrated the spleen. The importance of ascertaining information on this subject is evident when one recollects that one of the standard methods of measuring blood volume in man and animals is based on the administration of a known volume of carbon monoxide, and the estimation of the quantity of that gas in unit volume of blood. If the carbon monoxide penetrates to the hæmoglobin in the spleen there will be a less quantity in the blood than would otherwise be the case, and the blood volume as calculated by this method would be larger. Moreover, it was observed by the recent expedition to Peru (1) that the blood volume, as calculated by the carbon monoxide method, underwent considerable changes with the temperature of the atmosphere in which the subject was living; being greater in tropical temperature than in more temperate climates. This observation was subsequently confirmed by experiments in a glass chamber in which the air was artificially heated. In neither case was the change in blood volume simply due to increased plasma. The hæmoglobin as measured increased also, and at a rate which seemed too great to be accounted for by fresh formation of the pigment. Some investigation was therefore desirable as to whether there could be any considerable number of red blood corpuscles in the body to which the carbon monoxide did not reach during a determination but which could be thrust into the blood if circumstances demanded.

The first series of experiments performed were upon the spleens of a number of rats of which the blood volume was being determined for another purpose by one of us in collaboration with Dr Duncan Scott. These rats were given about ·5 c.c. of CO apiece, administered at first in high concentration (about 3 p.c.), the rats breathed the mixture of CO and oxygen for 5 to 10 minutes, they were then decapitated, the spleens were excised, cut up under water or a very dilute ammonia and the pig-

ment extracted was observed in the Hartridge Reversion Spectroscope, as also was a portion of the blood from the general circulation. The figures show that while there was a high percentage of CO in the blood, 30–40 p.c., the hæmoglobin dissolved from tho spleen was almost free from that gas, containing less than 5 p.c.

The previous observations on this subject are few and apparently somewhat at variance. Haldane has told me in conversation that he found CO hæmoglobin in the spleens of animals which have died with poisoning by carbon monoxide. But his observations show that it is not invariably present under such circumstances, and Heger suggests that CO is present in smaller amounts in the spleens of animals which have been exposed for a short time to a high concentration than of those which have been exposed to a low concentration but for a longer time. We therefore undertook a series of systematic observations for the purpose of ascertaining the relative saturations of tho hæmoglobin in the general circulation, and of that in tho spleens of rats exposed to the gas for varying lengths of time. So far as the experimental details of apparatus, etc., were concerned one experiment differed littlo from another in principle and the description of two will suffice for the whole except where otherwise stated.

(*a*) *Experiments in which the gas was inhaled for varying lengths of time.* In preliminary experiments, a given number of rats, usually six, were placed in a given concentration of gas for a given time which varied in different experiments from five minutes to an hour. In the different experiments of this series, the concentration was the same, ·6–·7 p.c., or as nearly the same in each experiment as possible. The rats were killed at the end of the experiment and the percentage of CO in the blood of tho general circulation compared with that in the hæmoglobin from tho spleen. This series quite agreed with the more perfect experiment which we shall now describe. In the chamber which was of six cubic metres capacity were enclosed one observer and twelve rats, two in each of six cages. The observer breathed not the air in the chamber, but the external air which was lead to him through rubber tubing connected with outlets in the wall of the chamber, valves and a mouthpiece. When the observer and the animals were enclosed the door of the chamber was shut, not to be re-opened till the end of the experimont. The door leads into an air-lock and in the door is a small hatch with double doors, large enough to receive a rat cage. Thus the rats can be handed out of the chamber without any sensible fall in the concentration in the chamber. The experiment commences when the carbon monoxide is put in from outside.

It is forced in from a flask, or if necessary two flasks of known volume by water displacement: this process takes about a minute and the time measurements are counted from the moment when half the carbon monoxide is judged to be in the chamber. The mixture of the CO with the air of the room is effected by a fan which is kept running throughout, but at the commencement the observer can help effectively by walking about and waving a newspaper or some flat object. At stated intervals the rats are handed out of the chamber, immediately preceding which they are stunned. Their throats are cut and 40 cubic mm. of blood are diluted in about 2 c.c. of ammonia (1 c.c. in 500). The dilution is done in a very subdued light and the test-tubes which contain the dilute blood are kept in the dark. They are about 2·5 c.c. capacity so that when corked up they have only a trifling air space above the fluid. This fluid is used for examination with the reversed spectroscope. The rats are set aside. Samples of the gas in the chamber are taken at the commencement and end of the experiment for analysis. The chamber was found to maintain the concentration satisfactorily.

Fluid from the spleen was obtained in the following way. The spleen was taken out, weighed, and placed under about 3 c.c. of dilute ammonia. It was then rapidly cut up under the ammonia and pressed with the fingers. There is no difficulty in obtaining pigment sufficient to be seen in the spectroscope. This dilute pigment gives a fair sample of the hæmoglobin of the pigment in the spleen pulp, for a second sample, which may be obtained frequently from the same spleen fragments, gives a CO content identical with the first. The spleen extract, unlike the dilute blood, is never quite clear. This we have found to introduce a small error amounting to about two divisions on the scale of the Hartridge spectroscope. Thus if a rat be taken which has not breathed CO and its blood be compared with its spleen extract, the latter will have an apparent CO content of − 5 p.c. or thereabouts. As the turbidity differs in different extracts it is not easy to make any appropriate numerical correction, but we found a satisfactory solution of the difficulty in the addition of a trace of milk to the dilute blood. The quantity of milk necessary is of course almost infinitesimal, but it can be graduated so as to imitate the spleen extract pretty exactly.

There are then two sources of error in the spectroscopic examination of the spleen fluid each of which tends to lower the apparent percentage of CO as compared with that in the dilute blood. The first of these is any loss of CO which may take place from exposure of the spleen extract, the econd is the turbidity. It will appear later that the spleen fluid

usually contains less CO than the blood and therefore the errors are such as to exaggerate the difference between the two. We therefore altered the plan of the experiment as follows:

(*b*) *Experiments in which the gas was exhaled for varying lengths of time.* The twelve rats were put in a chamber, in a certain concentration

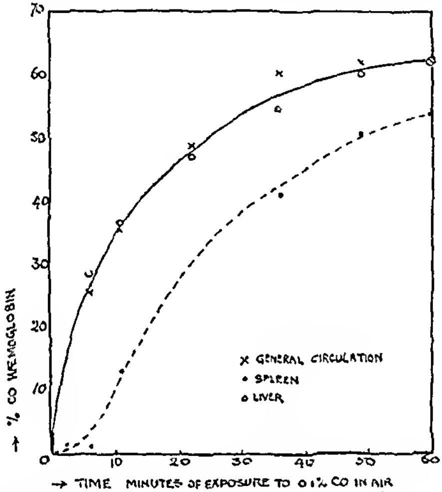

Fig. 1. The percentage of CO hæmoglobin is in each case the mean of that in two rats. The variations from the mean were respectively:

Blood	... ...	1·5, 0 5, 1, 0, 0 p c. HbCO
Spleen	... ...	0 5, 3 0, 1, 3, 3 „

The percentage was calculated from the displacement of the α band in divisions of the spectroscope, the mean of a number of scale readings being taken. For example, in Rat 6:

5 scale readings of blood varying from 31·5 to 34	... ...	mean	32 9
10 „ „ O_2Hb „ „ 17·5 to 21	... ...	„	19 5
Displacement of α band in divisions of spectroscope	...		13·4[1]
5 scale readings of blood of spleen varying from 21 to 25	... ...	mean	23·5
10 „ „ O_2Hb and milk (see text) varying from 16·6 to 21	...	„	18 6
Displacement of α band in divisions of spectroscope	...		4 8[1]

[1] This figure when applied to the calibration curve of the spectroscope (such as that given by Hartridge, This Journal, 44, p. 12, 1912) gives the percentage of COHb which is plotted on the ordinate.

of CO for an hour. By this time their bloods and spleens were almost in equilibrium with the gas. They were then all taken out and killed in pairs at varying intervals of time. The bloods and spleens were examined in the same way as in the preliminary series. The concentration of gas used was never sufficient to kill, or even seriously to interfere with the condition of the rats.

It need only be added that in some of the experiments, extracts of the liver, and kidney, were made for comparison with that of the spleen. The kidney extract proved incapable of accurate examination because the bands did not remain constant in position. Presumably some oxidative process was taking place which interfered with the oxyhæmoglobin bands. The liver extract gave satisfactory results and so furnished a useful control.

The results obtained may be gathered with sufficient accuracy from the figures. Fig. 1 shows an experiment of type (*a*) in which the percentage of CO in the blood, the spleen extract and the liver extract are plotted against the time in minutes. There is no appreciable difference in CO content between the lower extract and the blood of the general circulation—but the spleen extract only acquires its hæmoglobin much more slowly so that as much as half an hour may elapse between the time at which the blood in the general circulation has a certain content, say 53 p.c. saturation and the time at which the general mass of hæmoglobin in the spleen acquires the same CO content.

The same story is told by the experiments of type (*b*). Fig. 2 shows such an experiment. The concentration gas used proved to be slightly greater than that used in Exp. 1, giving the blood a percentage saturation of 67 p.c. instead of 62 p.c. CO. The first part of the figure shows the data from Fig. 1 adapted slightly to meet the slight difference in concentation. It therefore shows the rate at which the rat and its spleen were presumed to have acquired their CO. The second half (from the first hour onwards) shows the course of the actual experiment. It lasted four hours, at the end of which the two lines were within the region of experimental error of one another and the curve was very flat so that at this point the diagram became valueless. It is clear the lag in the exit of CO from the spleen may be an hour and a half as is shown at the point of 20 p.c. saturation.

The interpretation to be put on these diagrams is that as follows. One may consider the facts from one of two points of view. (1) The blood corpuscles may be regarded as in circulation in some portions of the spleen but stagnant in other parts, and the CO may be supposed to go

by diffusion from the circulating corpuscles to the stagnant ones in which case, the time taken up by the diffusion process accounts for the lag, or (2) one may suppose that all the CO is contained in corpuscles which

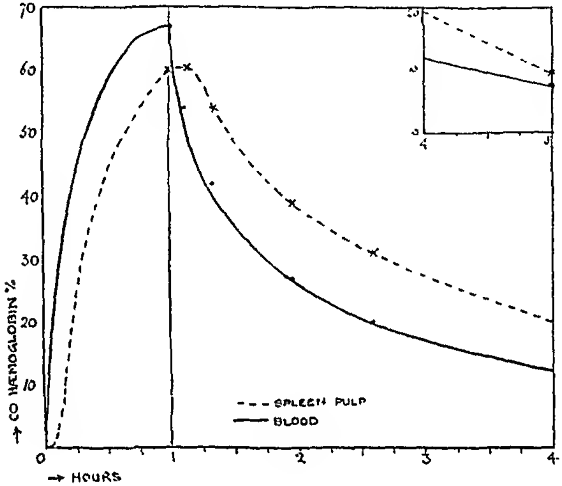

Fig. 2.

circulate and that there is no diffusion from one corpuscle to another. It is not suggested that either of these views is correct, they are merely the extremes between which the actual truth probably lies. According to the first view the time which some at least of the corpuscles are retained in the spleen is infinitely long, according to the second it is at least an hour and a half, for clearly if the average composition of the whole mass of corpuscles in the spleen pulp is the same as that of the blood ninety minutes previously, many of the corpuscles must have been there considerably longer than that time.

It would appear, then, that much of the spleen pulp is a sort of "backwater" in which large quantities of corpuscles can be held. In this respect it differs from the liver in which if the corpuscles stagnate to any extent, they do so in such close contact with the general circulation, that the time taken for the CO to diffuse into them is inappreciable by the method used in the above research.

CONCLUSION.

When the hæmoglobin of the spleen pulp, in rats breathing ·06–·1 p.c. CO, is compared with that of the general circulation, there is a lag between the percentage of CO hæmoglobin in the general circulation and that in the spleen pulp which, it is shown, may attain thirty minutes. A similar lag which may attain ninety minutes in demonstration in rats which have been breathing CO and are exhaling this gas.

The expenses of the research have largely been borne by the Hæmoglobin Committee of the Medical Research Council.

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NOTE ON THE EFFECT OF EXTERNAL TEMPERATURE ON THE CIRCULATION IN MAN. By JOSEPH BARCROFT (Cambridge) AND E. K. MARSHALL, Jr. (Johns Hopkins University).

(From the Physiological Laboratory, Cambridge.)

Uyeno(1) showed in cats under urethane, that a rise in the temperature of the surroundings, whether they were air or water, caused a considerable increase in the minute volume of blood in circulation, whilst exposure to cold had the opposite effect. These results were accompanied by changes in the body temperature and metabolism so considerable, that the circulatory effects might with some reason be regarded as secondary. One idea in starting the present research was to test whether exposure to heat or cold in man had similar effects to those demonstrated by Uyeno on the cat, though the accompanying change in body temperature in man is very slight. It is clear, of course, that the circulation in the skin varies with the temperature of its surroundings. If, however, the circulation in the skin is normally increased by A litres per minute it is not clear whether the total minute volume is increased to the same extent, or whether other parts of the body are deprived of A litres per minute in order that the blood may be diverted to the skin. So far we have only obtained a partial answer to this question, as other interesting points arose which we shall now describe.

Method. Our method of measuring the minute volume in man is based on the "triple extrapolation method" of Redfield, Bock and Meakins(2). We commenced by using this method but soon found that there were numerous cases in which the three extrapolations did not meet in a single point. This and other reasons led us to modify the method.

By the method of triple extrapolation the minute volume may be calculated from tension either of oxygen or carbonic acid pressures and consequent oxygen or carbonic acid content of the mixed venous blood. If the calculation be made correctly, whether on the basis of the oxygen or that of the carbon dioxide, the same answer for the minute volume should be obtained. If the estimates made from the two sets of data (oxygen and carbon dioxide) differ, one or both must be wrong. Under any given circumstances, therefore, there can then be only one possible

in the centre of which is placed the observed point. If a line be drawn touching the left margin of the square round A_1 and the right margin of the square round A_2 and another touching the right margin of A_1 and the left margin of A_2, their intersection with the equilibrium line shows the limits of accuracy of the observed venous tension of oxygen. In Fig. 3 this would be about 1·4 mm., *i.e.* the true point of oxygen tension in the venous blood may be 0·7 mm. on either side of the point at which the intersecting line cuts the equilibrium line. In some cases the error is greater for it will be clear that the greatest accuracy is only to be obtained by arranging the experimental details so as to secure the maximum separation of the points A_1 and A_2 and the closest proximity between A_2, the equilibrium line and the optimum slope of the intersecting line. The desired result is most nearly obtained by extending the time which elapses between the two points to the longest admissible interval. This in rest is unfortunately only about ten seconds, whilst in exercise it would be even less.

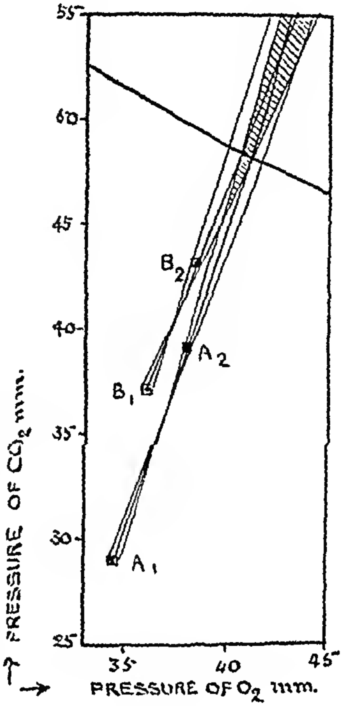

Fig. 3.

Control experiments were designed to see whether the degree of accuracy which appeared possible theoretically could be obtained in practice. In the following experiment in which four intersecting lines were determined one after the other, the subject had been seated for one hour before the determinations were made, in order to get his circulation into a nearly stable condition. The determinations were made within 15 minutes with the following results:

	A	B	C	D	Mean
Oxygen tension mm.	39·6	40·2	40·5	40·0	
% saturation	68·9	70·1	70·6	69·8	
Oxygen difference between arterial and venous blood in c.c. per litre ...	51·5	49·4	48·4	49·9	
Minute volume of blood in litres ...	6·02	6·29	6·42	6·23	6·24
Systolic output (pulse 70) c.c. ...	86	90	92	89	89

Uniformity was obtained to the extent of about 230 c.c. from the mean value of under 4 p.c. of the minute volume.

Another point of which we were anxious to make sure was that the addition or otherwise of carbon dioxide to the nitrogen in the bag made no difference to the observed minute volume. Naturally when carbon dioxide is added to the alveolar air, determinations show a higher pressure of that gas, and they also show a higher pressure of oxygen (that of B_1 Fig. 3 being higher than that of A_1 and that of B_2 than that of A_2) but the lines drawn through the A and B points respectively intersect the equilibrium line within half a millimetre's oxygen pressure of one another. This is shown in Fig. 3 where the shaded portion is within the experimental error of both the A determinations (without carbon dioxide in the bag) and the B determinations (with carbon dioxide).

The normal blood flow. Considerable discussion has taken place as to what may be regarded as the normal blood flow in man. The views of most observers tend to fall into two groups, Krogh and Lindhard (3) believing it to be 3 to 4 litres per minute, whilst Douglas and Haldane (4) regard it as about 7 litres. At first sight the point may not seem to be worth stressing as Krogh and Lindhard (3) have observed normals higher than the figure given above whilst Douglas and Haldane have observed cases which they regard as exceptional which approximate to Krogh's figures, and minute volumes as low as 2·5 litres have been reported. Nevertheless behind this discussion there lies a more fundamental point. The normal pulse-rate may be regarded as capable of something of the order of a threefold increase during exercise. If the normal flow at rest is 7 litres and if this also were increased threefold during exercise, the resulting 21 litres could conceivably transport about 4000 c.c. of oxygen for the use of the body. Over this range then the output of the heart at each beat might remain constant, but if on the other hand the normal "minute volume" is but 3 litres and this be increased threefold less than 2000 c.c. of oxygen per minute could be carried from the lungs—a quantity much less than what we know to be actually used by the body. Thus the 7 litre normal is consistent with a doctrine of the uniformity of systolic output put forward by Yandell Henderson in 1906 (5) as the result of experiments on dogs and recently given considerable support by Douglas and Haldane, while the 3 litre normal is inconsistent with that theory. It is therefore less the "normal" than the "minimal" minute volume which is of interest. The above authors do not define with any great precision the circumstances which they regard as "normal."

In the experiments which follow we have felt at liberty to apply that term to the "minute volume" of a person who has been sitting for

15 minutes or more quietly in a chair, for the works of Campbell, Douglas and Hobson (6) and also of Lupton (7) show that but a few minutes suffices, after muscular exercise for the metabolism to return to its normal level. Moreover Lindhard in a recent paper has shown that after muscular exercise the blood flow falls rapidly within the first few minutes. At the end of about 15 minutes it settled in the subjects whom he observed apparently to a nearly constant level. His figures only extend to the 24th minute after exercise.

The following determinations of the minute volume measured not less than 15 minutes after the commencement of rest, show that it is capable of great variations, and that while the majority of observations on Barcroft are of the order of 7 litres those on Marshall are for the most part about half that amount. So far our results show the same sort of divergence as those of other authors (see Krogh and Lindhard (3)). But a bald statement that Barcroft's minute volume is about 7 litres while Marshall's is about 3 only gives a partial picture of the difference between the two, for by sufficient rest Barcroft's also may be reduced to the lower figure. The difference between the vascular systems of the two men seems to lie in the fact that Marshall's minute volume settles rapidly, *i.e.* within 15 minutes, to a nearly steady value, while Barcroft's gradually falls for at least two hours after taking his seat on occasions to something near the same final value. In neither case, however, is our experience quite the same as that of Lindhard who says "At rest the utilisation is rather small and of fairly constant magnitude" for in the case of Marshall the utilisation though fairly uniform is not small while in the case of Barcroft the utilisation—at first small is not at all constant, for it will be clear from the figures which are given below that the change in minute volume is associated with altered utilisation rather than altered metabolism.

Observations in minute volume—sitting.

	Barcroft				Marshall		
	A	B	C	D	A	B	C
Time from taking seat, minutes	65	60	90	120	68	78	131
Metabolism in c.c. of oxygen per minute	301	301	296	291	274	281	261
Oxygen tension in venous blood, mm.	45	42	40·6	38	35·8	34	33·3
Utilisation c.c. per 100 of blood	20·0	24·5	25·3	29·0	28·5	42·5	44·3
Minute volume in litres	7·9	6·7	6·2	5·3	3·8	3·8	3·2
Pulse rate	68	62	66	61	73	69	63
Systolic output in c.c.	116	108	93	87	52	55	51

The effect of cold. The effect of cold more extreme than that produced by sitting in the laboratory in cold weather was tested in the cold storage laboratory, for the use of which we are indebted to Mr W. B. Hardy.

Our experiments were planned as follows. The subject sat for 15 minutes at 15° C. just outside the cold room, at the end of which his minute volume was measured. Then he walked with the least possible effort about 10 yards into the cold passage (temperature $-1°$) and at the end of 15 minutes his minute volume was again determined. Barcroft proved much less sensitive to cold than Marshall. It was necessary for him to remove most of his clothing in order to become sufficiently chilled in the time. The criterion of coldness at which we aimed was that at the end of the 15 minutes the tendency to shiver should be well marked. It was found easy to observe whether or not there was a tendency to shiver by holding the knees a little apart as one sat, and seeing whether there was a tendency for them to come together. The shivering in our experiments never amounted to movement so obvious that it could be observed by another person. In the control experiments the subject sat as before during the first 15 minutes, then arose and walked once round the room to imitate the journey into the cold store and resumed his seat for a second 15 minutes. The general effect was the same in all experiments differing, however, in degree and was rather unexpected. The minute volume increased, as did the metabolism, whilst the pulse rate fell. The two phenomena combined produced a very considerable increase in the systolic output. In pathological cases such as paroxysmal tachycardia (Barcroft, Bock and Roughton (9)) and mitral stenosis (Meakins, Dautrebande and Fetter (10)) the pulse has been observed to accelerate whilst the minute volume decreases. This, however, might be attributed to the insufficiency of the heart and be regarded as of the essence of the disease. It is doubly important therefore to find a set of conditions in health in which the pulse and the minute volume go in opposite directions.

Effect of exposure for 15 *minutes to* $-1°$ C.

A = room temperature. B = cold store.

Subject	Metabolism c.c. O_2 per min. A	B	Minute volume litres A	B	Pulse A	B	Systolic output c.c. A	B
Barcroft (1)	348	412	6·7	7·6	73	60	91	124
(2)	350	410	7·4	9·3	76	60	99	154
(3)	328	364	6·5	8·6	73	57	89	151
Marshall (1)	269	408	5·6	10·9	82	74	68	148
				Controls				
	A	A	A	A	A	A		
Barcroft (1)	325	319	7·6	5·5	69	67	104	82
(2)	289	300	6·4	6·8	68	64	94	105

In the table given it will be observed that the minute volume always goes in the same direction as the metabolism, *i.e.* goes hand in hand with

The particular question which leads us up to this enquiry was one raised by the recent high altitude expedition to Peru. In the rather limited data obtained by that expedition on its journey into the tropics two facts appeared to emerge; (1) that the minute volume for the body did not increase in passing from a temperate to a tropical climate, and (2) that the blood volume increased and that to such an extent as to involve an increased formation of corpuscles. If the circulation through the skin amounted, as would appear from the above determinations, to about 3 litres, the deduction would be that the circulation through the body was reduced by that amount. A possible stimulus to the blood formation would then be the deprivation of the blood forming organs of part of their oxygen supply. Thus the new blood formation would fall into line with the general principle that anoxæmia causes an increased activity of the red marrow.

The inconsistency of the systolic output. Throughout the experiments detailed above, but more especially those upon cold, there is a considerable variation in the systolic output amounting in some cases to over 100 p.c. in a relatively short time. As mentioned above, Yandell Henderson put forward the general thesis that the systolic output is constant and this received a general support from the work of Douglas and Haldane. Our observations clearly cut right across any such generalisation, nevertheless there is less conflict than appears at first sight between our work and that of Douglas and Haldane. The conflict is only when their results obtained over a *limited* range of minute volumes—and a range in which we have not worked—are expressed as a general principle. Douglas and Haldane obtained a constant systolic output with minute volumes of from 7 to 8 litres upwards, whilst we have worked with minute volumes of from 7 to 8 litres downwards. It should be noted indeed that two persons are cited (Adolf and Peskett) in Douglas and Haldane's paper with a resting minute volume of between 4 and 6 litres. These show an increased systolic output when the minute volume is increased. Indeed the meagre data in Adolf show a constant systolic output above a point somewhere between 6 and 11 litres (*op. cit.* p. 86).

Possibly at very high minute volumes the systolic output alters again. But while it may be argued that the systolic output is constant within certain limits of minute volume there is at present no evidence to show that it is constant within given limits of heart beat. In the experiments on heat we have only driven the pulse rate up to about 80 but with very various results on the systolic output.

Summary and Conclusions.

1. A method is described by which the pressure of oxygen in the mixed venous blood can be determined more simply and quickly than by the triple extrapolation method of Redfield, Bock and Meakins.

2. The minute volume measurements based on this method agree with those of other workers in showing great differences in different persons. This divergence is to some extent due to the length of time which the different individuals require in order to reach a steady state.

3. In man exposure to cold till the point of shivering is nearly reached increases the minute volume whilst it slows the pulse, thus producing a large alteration in the systolic output.

4. Exposure to warmth in the case of a person whose minute volume adapts itself quickly, raises the same by 3–4 litres—a quantity which probably is a rough measure of the blood flow through the skin.

5. At low minute volumes below 8 litres the systolic output is susceptible of great alterations.

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(4) Douglas and Haldane. This. Journ. 56. p. 69. 1922.
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(11) Meakins and Davies. Journ. Path. and Bact. 22. p. 451. 1920.
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NOTE. Whilst this paper was in the Press we found that Lindhard (Pflüger's Arch. 161. p. 294. 1915) has studied the effect of hot and cold baths on the minute volume in man. Using the nitrous oxide method of Krogh and Lindhard(3), he found an increase in the minute volume in three out of four subjects exposed to a bath of 41° to 42° C.; and in two out of four subjects a decrease when put in a bath at 15° to 17° C.

APPENDIX.

Methods of calculating the carbonic acid pressure in the mixed venous blood, which corresponds to a given oxygen pressure—say 40 mm.

1. The oxygen pressure is assumed.

2. The oxygen dissociation curve for capillary blood such as is given by Haldane, Christiansen and Douglas, This Journ. 48. p 262 is required. The present example

will be worked out for Barcroft's blood. On his curve 40 mm. oxygen pressure corresponds to 66 p.c. saturation. This, then, is the oxygen saturation in the mixed venous blood.

3. The oxygen saturation in the arterial blood is assumed to be 96 p.c. The coefficient of oxidation is therefore $96-66=30$ p.c.

4. The hæmoglobin value was 105. Therefore the total oxygen capacity of the hæmoglobin was $\frac{105}{100}\times\cdot 185=\cdot 195$ c.c. The actual amount of oxygen which the hæmoglobin of each c.c. of blood gains in the lung is 30 p.c. of $\cdot 195=\cdot 056$ c.c.

5. In addition the plasma gains oxygen. The amount of oxygen in c.c. dissolved in 1 c.c. of venous blood at 37° C. and 40 mm. pressure is $0{\cdot}022\times\frac{40}{760}$ whilst that in 1 c.c. arterial blood is $0{\cdot}022\times\frac{100}{760}$. The gain is $0{\cdot}022\times\frac{70}{760}=0{\cdot}002$ c.c. (correct to the third significant figure).

6. The total oxygen gained by the blood is therefore $\cdot 056$ c.c. $+\cdot 002$ c.c. $=\cdot 058$ c.c.

7. The respiratory quotient measured from the expired air, ·80 c.c., the pure respiratory quotient is therefore ·76 (Methods of Gas Analysis, Haldane, Charles Griffin and Co., London, 1912; see table on p. 57). The CO_2 given out in the lung for each c.c. of blood was therefore $\cdot 058\times\cdot 76=\cdot 044$ c.c.

8. The CO_2 pressure in the alveolar air which is assumed to be that in the arterial blood was 40·2 mm.

9. The CO_2 dissociation curves for the reduced and oxidised bloods. It is necessary to find out the CO_2 content of arterial blood, which is 96 p.c. saturated with oxygen. The blood fully saturated with oxygen at 40·2 mm. CO_2 pressure contains 51·5 c.c. CO_2 per 100 of blood. Whilst fully reduced it contains 57·4 c.c.—difference $(57{\cdot}4-51{\cdot}5)=5{\cdot}9$ c.c. The actual content of the arterial blood at 40·2 mm. pressure is therefore $57{\cdot}4-5{\cdot}9\times\frac{96}{100}$ $\left(\text{or }51{\cdot}5+5{\cdot}9\times\frac{4}{100}\right)=51{\cdot}7$ c.c. CO_2 per 100 c.c. blood, *i.e.* 0·517 c.c. CO_2 per 1 c.c. of arterial blood.

10. The CO_2 content of 1 c.c. of venous blood is therefore $0{\cdot}517+0{\cdot}044=0{\cdot}561$.

11. It remains to find the partial pressure at which 1 c.c. blood contains 0·561 c.c. CO_2 when 66 p.c. saturated with oxygen; this is done by a process similar to that given in section 9 and is 47·1 mm. [For the processes described in sections 9 and 11 it is best once for all to construct a diagram giving the CO_2 dissociation curves for the oxidised and reduced bloods between 35 and 55 mm. pressure, and interpolating the curves for each 10 p.c. O_2 saturation between the two.]

VARIATIONS IN THE BLOOD CHLORIDES IN RELATION TO MEALS. Part I. By E. C. DODDS[1] AND K. SHIRLEY SMITH.

(*From the Biochemical Department, Bland-Sutton Institute of Pathology, Middlesex Hospital.*)

THIS paper forms one of a series of investigations into the body changes accompanying meals. In previous papers the variations in alveolar CO_2(1) tension in relation to meals have been described, and also the accompanying changes in blood CO_2 tension(2). It was shown by one of us (E. C. D.) in conjunction with McIntosh that as the alveolar CO_2 tension rose during gastric secretion the corpuscular CO_2 content also increased, but the plasma CO_2 content remained more or less constant. During the fall in CO_2 tension in the alkaline period of secretion there was a fall in corpuscular CO_2 content, the plasma again remaining more or less constant. No change could be detected in plasma alkali reserve or actual hydrogen ion concentration as measured by the hydrogen electrode.

It was thought that a study of plasma and corpuscles in relation to meals might yield some information on the mechanism of production of HCl in the gastric juice. That the corpuscles must play some very important part in the production of HCl is shown by their varying composition during gastric secretion as contrasted with the relative constancy of composition of the plasma during this period. The importance of the red blood corpuscles in transferring chlorine to and from blood plasma has been shown by several observers, notably by Haggard and Henderson(3) and by Dautrebande and Davies(4) on the hæmato-respiratory functions.

The present investigations were made into the chloride content of the blood in relation to meals. The subjects were all healthy students, on whom a fractional gastric analysis had been performed. By this means subjects with achlorhydria were excluded. Two series of experiments were carried out. In the first series the chlorides contained in the whole blood, plasma, and corpuscles were estimated before the mid-day meal and at varying intervals after it. Only two samples of

[1] Working on behalf of the Medical Research Council.

blood were taken from the same individual. In the second series blood chloride estimations were carried out in conjunction with fractional gastric analysis, three samples of blood being taken from each subject.

Method. Series I. Between 8 and 10 c.c. of blood were drawn from a vein of a fasting subject, and shaken with a crystal of oxalate to prevent clotting. About two-thirds of the blood was centrifuged at once to minimise possible extra-vascular changes in chloride-partition in the blood. The remaining third was estimated by Wetmore's method(5) for chlorides. Plasma taken after 10 minutes' centrifuging was then estimated. It was attempted also to measure chloride content directly in the corpuscles by a modification of Wetmore's method but it was found that the values obtained did not agree with those calculated from the whole blood and plasma readings taking the corpuscles as constituting 48 p.c. by volume of the whole blood. It also became apparent that certain experimental difficulties tended to make the corpuscle determination inaccurate; these determinations were therefore disregarded, and the calculated values used in their place.

The meal was taken as a rule between 1 and 1.30 and the second sample of blood drawn at times varying from 40 to 135 minutes after the beginning of the meal. By such a procedure it was hoped to demonstrate any changes occurring in chloride content and partition as a result of gastric, and possibly of the beginning of pancreatic secretion.

As control experiments, four subjects were investigated exactly as described above, except that no meal was taken between the drawing of the first and second sample of blood. One of the control subjects had, in a previous experiment, shown considerable variation in blood chloride as a result of a meal.

Method. Series II. The subject came to the laboratory at about 9.30 a.m., having taken no food since the previous evening. He then swallowed the œsophageal tube, resting juice being extracted from the stomach by means of a syringe every quarter of an hour; this juice was estimated for free HCl. With the stomach empty, blood was drawn and estimated as whole blood and plasma, as previously described, the corpuscle values being calculated. The subject then drank a pint of the test-meal recommended by Krohn and Reiss(6) and 10–15 c.c. of stomach contents were withdrawn at quarter-hour intervals thereafter; each sample was estimated immediately for free HCl and total acidity. When it was found that the height of the gastric HCl secretion was at hand a second sample of blood was taken and estimated as before. Finall , a third quantity of blood was taken about two hours after the

meal when the gastric secretion was drawing to a close and the stomach was practically empty.

Results. In Table I the experiments have been arranged in order of

TABLE I.

	Subject	Cl before meal in* Whole blood	Plasma	Cor-puscles	Time between meal and taking blood (mins.)	Cl after meal in* Whole blood	Plasma	Cor-puscles	
1	H. K. E.	·48	·605	·345	40	·47	·595	·335	1
2	B. M. R. J.	·50	·65	·463	40	·47	·50	·329	
3	R. W. D.	·485	·57	·393	40	·45	·565	·325	
4	W.	·5	·785	·101	55	·425	·625	·208	2
5	D. P. G.	·48	·61	·339	60	·49	·605	·365	
6	H.	·42	·575	·252	75	·42	·56	·268	
7	G. C. P.	·44	·58	·288	90	·45	·57	·320	
8	H. I. D.	·475	·57	·372	100	·465	·585	·335	3
9†	F.	·49	·59	·382	105	·48	·59	·351	
10	F. O. W.	·44	·565	·305	135	·425	·545	·295	

* Figures represent chloride as NaCl in gm. per 100 c.c.
† Subsequently found to be achlorhydric.

length of time intervening between the meal and the drawing of the second sample of blood. With regard to the chloride content of the whole blood it is seen that as a general rule the meal is followed by a fall which may amount to 16 p.c. of the initial quantity present. The chlorides of the plasma show a more uniform decrease, though in one instance (No. 4) it was rather more than 20 p.c. This subject, however, had an unusually high plasma content before the meal. No. 8 is the only exception, and since he showed a fall in a subsequent experiment with the œsophageal tube, it may be concluded that in this case some error has crept in. But it is in the corpuscles that the most striking changes in chloride content are found. In this respect the experiments may be divided into three groups: (1 to 3) with a time interval of less than 45 minutes; (4 to 7) with an interval of 45–90 minutes; and (8 to 10) with an interval of 90–135 minutes. In the first of these groups is shown a decided fall in chlorides, in the second a marked increase (approximately 8 p.c.), and in the third group a fall not quite as great as in the first group.

In Table II the results of the combined blood and gastric analyses are shown. It can be seen that in the specimen of blood removed at the height of gastric secretion there is a fall in plasma and a rise in corpuscular chloride content. The whole blood chloride content shows a steady fall. At the termination of the curve of gastric secretion, the plasma chloride content shows a partial recovery to its former value, whilst the corpuscular value shows a fall to below the initial value. This is well shown in cases 1 and 2 of Table II. Case 3 is one showing a prolonged gastric secretion.

The second and third samples of blood were drawn while the curve of secretion was still on the rise. The values obtained, therefore, correspond

TABLE II.

1. G. C. P. Meal at 10.55 a.m.

Time	10.0	10.30	10.55	11.15	11.30	11.45	12.5	12.20	12.35	12.50
Free HCl in c.c. N/10	0	0	0	15	40	60	30	36	28	20

	Cl. as gm. NaCl per 100 c.c. Whole blood	Plasma	Corpuscles
Blood I taken at 10.35	·46	·575	·335
Blood II taken at 12.0	·46	·55	·363
Blood III taken at 1.5	·445	·57	·310

2. H. I. D. Meal at 10.35 a.m.

Time	10.15	10.30	10.50	11.5	11.20	11.35	11.50	12.5	12.20	12.35
Free HCl in c.c. N/10	10	24	3	12	50	74	76	56	30	44

	Cl. as gm. NaCl per 100 c.c. Whole blood	Plasma	Corpuscles
Blood I taken at 10.35	·50	·63	·359
Blood II taken at 11.55	·49	·605	·365
Blood III taken at 12.50	·485	·61	·349

3. A. M. F. Meal at 11.15 a.m.

Time	10.10	10.30	10.45	11.0	11.20	11.35	11.50	12.5
	12.20	12.35	12.50	1.5	1.20	1.35	1.50	
Free HCl in c.c. N/10	0	28	30	43	0	0	0	17
	36	52	58	58	60	65	70	

	Cl. as gm. NaCl per 100 c.c. Whole blood	Plasma	Corpuscles
Blood I taken at 10.15	·52	·63	·401
Blood II taken at 12.30	·51	·63	·380
Blood III taken at 1.57	·51	·615	·396

to those of 1, 2, 3 in Table I, and hence show a fall in corpuscle and plasma chloride. The final specimen shows a marked rise in corpuscular chloride content.

The readings of the four control experiments are given in Table III.

TABLE III. Control experiments.

No.	Subject	Cl in* Whole blood	Cl in* Plasma	Cl in* Corp.	Cl in (1 hour later) Whole blood	Cl in (1 hour later) Plasma	Cl in (1 hour later) Corp.	Cl in (2 hours later) Whole blood	Cl in (2 hours later) Plasma	Cl in (2 hours later) Corp.
1	E. C. D.	·48	·61	·339	·48	·61	·339	·48	·61	·339
2	A. L. W.	·47	·59	·340	·472	·59	·344	·47	·60	·329
3	A. N. K.	·50	·78	·197	·50	·78	·197	·50	·78	·197
4	B. M. R. J.	·47	·59	·340	·47	·58	·351	—	—	—

* Figures represent chloride as NaCl in gm. per 100 c.c.

From them it may be seen that the fluctuations in blood chloride content of whole blood, plasma and corpuscles in the absence of a meal are

extremely small, and well within the limits of experimental error. This fact is still better demonstrated by a comparison of the figures for Subject 4 with his figures in Table I (No. 2) where a meal intervened between the drawing of the samples of blood.

Discussion. It was found by Gürber(7) that as a result of increase in the CO_2 tension in the plasma, the alkalinity of the latter increases. This, he found, was due to a migration of Cl into the blood corpuscles, the kation being left to form bicarbonate with the CO_2 of the plasma. Further, it has been shown by one of us (E. C. D.) that such a condition of increased blood CO_2 tension, as evidenced by increased alveolar CO_2 tension, occurs after a meal during the period of gastric secretion. It would be expected then at this time to find also an increase in the chloride of the corpuscles, and this fact is demonstrated by the figures given in Table I (4 to 7).

Within the first 40 minutes following the meal, a sudden demand for Cl is made on the blood, with the result that the chlorides fall universally. As gastric secretion proceeds and the blood CO_2 tension rises the plasma must not only give up Cl to the gastric glands but also to the corpuscles which become loaded. The loss of Cl from the plasma is possibly made up in part from the chlorides of the tissue spaces and re-absorption from the digestive tract. Between $1\frac{1}{2}$ and $2\frac{1}{4}$ hours following the meal, gastric secretion is coming to an end, and pancreatic secretion with its concomitant fall in blood CO_2 tension is setting in. The last group in Table I (8 to 10) shows how the corpuscles unload their excess Cl in response to falling CO_2 tension in the blood.

How far the increase in chloride content of the corpuscles plays a part in the mechanism of formation of the gastric HCl cannot as yet be said. It may be that this redistribution is merely a necessary result of the changes in CO_2 tension, in combination with the property of permeability possessed by the cell. However, in the light of a great body of work which demonstrates the immense importance of the corpuscles as active agents in the various functions of the blood, it is probable that the chloride changes in the corpuscles should be regarded as a step in the very complicated and as yet unknown mechanism by which HCl is formed and secreted with the gastric juice.

Conclusions.

1. The chloride contents of whole blood, plasma and corpuscles were investigated at various times after a meal with the following results:

(*a*) During the first 40 minutes, the whole blood, plasma, and corpuscle chloride content showed a fall.

(*b*) During the period between 45–90 minutes after a meal, the whole blood chloride content showed a recovery to its normal figure, whilst the corpuscles showed a marked increase above the normal value. The plasma remained about the same as in the former group.

(*c*) From 90–135 minutes following the meal the whole blood chloride content had again fallen slightly. The plasma and corpuscle values showed a partial return to the initial values.

2. By combining fractional gastric analyses with blood chloride estimations, the first period was shown to correspond with the commencement of gastric secretion, the second with the height of gastric secretion, and the third with termination of gastric and probably the beginning of pancreatic secretion.

3. A series of control experiments was performed in which the meal was omitted. No change in blood chlorides could be demonstrated.

4. In accordance with the increased alveolar CO_2 tension during the period of gastric secretion, it was found that the corpuscles increased their chloride content, the latter decreasing again with falling alveolar CO_2 tension. This is in agreement with the so-called "Hamburger effect."

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THE HEAT OF COMBUSTION OF GLYCOGEN IN RELATION TO MUSCULAR CONTRACTION

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HARTREE and HILL(1) in a recent paper have given a full analysis of the heat evolved in the various stages of isometric muscular contraction, and applying Meyerhof's(2) figure of 370 cals per gm of lactic acid formed under anaerobic conditions were able to give absolute values for the different phases of activity The following figures are pertinent to the investigation described below

Total anaerobic heat	370 cals	per gm	of lactic acid
Total initial heat	296	,,	,,
Delayed anaerobic heat	74	,,	,,

It is of interest to compare these figures with those calculated from the heats of combustion of glycogen and lactic acid, and the heat of neutralisation of lactic acid by salts such as phosphates and carbonates or by alkali-protein[2]

If the accepted chemical view be correct, it should be possible to account for the initial and delayed anaerobic heats with considerable accuracy by the heat changes taking place in the decomposition of glycogen and the neutralisation of the resulting lactic acid

For the purposes of the calculation there are available

(*a*) the heat of combustion of lactic acid in dilute solution, determined by Meyerhof(3) to be 3601 cals per gm ,

(*b*) the heats of neutralisation of lactic acid by salts and alkali-protein, shown by the same worker(3) to be 19 cals and 138 cals per gm respectively, and

(c) the heat of combustion of glycogen for the solid anhydrous material, which had been determined by Stohmann and Schmidt(4) who obtained the value 4190 cals per gm

Since 0 9 gm of anhydrous glycogen yields 1 gm of lactic acid, the

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[2] The term alkali protein is used throughout the paper to signify the sodium and potassium salts of protein

heat of combustion of the glycogen forming 1 gm. should be $0 \cdot 9 \times 4190$ cals., *i.e.* 3771 cals. As the resulting gram of lactic acid has a heat of combustion of 3601 cals. the heat liberated in its formation from glycogen could only have a maximum value of 170 cals. That this value is a maximum must follow unless the hydration and solution of glycogen are endothermic processes; from analogy, however, it is probable that the value would actually be too large, since in similar substances the hydration and solution are definitely exothermic.

If it be assumed that the lactic acid is neutralised immediately on formation by the alkali-protein, a value is obtained of $(170 + 138)$, *i.e.* 308 cals. for each 1 gm. of lactic acid formed. This value would explain the initial heat, but would leave the delayed anaerobic heat entirely unaccounted for. It is necessary, therefore, if the figures on which the calculation is based be assumed to be correct, to postulate some further process so far unknown, which takes place in anaerobically contracting muscle at a comparatively slow rate for some time after the formation and neutralisation of the lactic acid. It is difficult in view of the chemical evidence to imagine what form such a reaction would take.

It was suggested to the author by Mr A. D. Ritchie that an investigation of the heat of wetting and solution of dry glycogen might throw some light on this discrepancy. The work was undertaken, and ultimately developed into a redetermination of the heat of combustion of glycogen. Those details of the investigation which are purely chemical will be given in another place, after some further points of interest in connection with the chemistry of glycogen have been elucidated. The present outline is intended merely to indicate the points of physiological interest.

The glycogen was prepared from sea water mussels (*Mytilus edulis*) which provide an easily purified and plentiful source of the material. The purification process consisted of heating on a water bath with 60 p.c. potash, and repeated precipitation with alcohol. The final product was ash free, fat free, and contained only minute traces of nitrogen. When the product was dried in an air oven at 110° C., a method generally adopted by previous workers, it was found impossible to reach a stage where the preparation ceased to lose weight. As an alternative, the glycogen wet with water and alcohol was dried by distilling off the constant boiling mixtures of alcohol-benzene-water and alcohol-benzene or water-benzene as the case might be, after the manner described by Atkins and Wilson (5). The resulting product after the benzene had been removed in a current of air, was an amorphous white solid stable in air, which

on ultimate analysis proved to be the hydrated form $(C_6H_{10}O_5.H_2O)_n$ and not the anhydrous substance $(C_6H_{10}O_5)_n$.

Considerable interest attached to the further drying of the hydrate. When it was dried over calcium chloride *in vacuo*, the half hydrate $(C_6H_{10}O_5.\frac{1}{2}H_2O)_n$ resulted. A similar observation was made over fifty years ago by Bizio(6). Drying in an air oven at 110° C. does not give complete dehydration. After ten days' continuous drying the glycogen still contained 5 p.c. of its water of hydration, and the rate of drying had become almost negligible. The product was hygroscopic, picking up water rapidly on exposure to air. These observations are in agreement with those made by Harden and Young(7), who showed that only by heating to 100° C. over phosphorus pentoxide *in vacuo* could glycogen be rendered completely anhydrous.

As previous workers had prepared "anhydrous" glycogen by drying in an air oven, it seemed probable that their figures for the heat of combustion would be too low, owing to the unsuspected presence of water in the purified glycogen used. It was decided, therefore, to repeat the determination of the heat of combustion, in addition to the determination of the heat of wetting and solution. The total heat of wetting and solution of the hydrated glycogen was determined, the average value obtained in three experiments, which agreed within 1·5 cals., being 9 cals. per gm. For the heat of combustion a value of 3883 cals. per gm. as the average of five concordant experiments was obtained. Hence the value for the heat of combustion of hydrated glycogen in solution is 3874 cals. per gm. This is about 100 cals. higher than that deduced from the determination of Stohmann and Schmidt.

Employing Meyerhof's value for the heat of combustion of lactic acid the heat liberated on the conversion of 1 gm. of glycogen to 1 gm. of lactic acid works out at (3874–3601), *i.e.* 273 cals. If it be assumed that the neutralisation of lactic acid on formation is carried out entirely by salts, then the heat liberated during contraction and relaxation would be (273 + 19), *i.e.* 292 cals. per gm. of lactic acid formed. This figure is in remarkable agreement with the total initial heat as determined by Hartree and Hill, viz. 296 cals.

Meyerhof(2) and Hartree and Hill(1) have shown that salt buffering alone is inadequate to deal with the large amounts of lactic acid formed during continued muscular activity. It would seem reasonable therefore to assume that the immediate salt buffering is only temporary in character, and replaced by the more efficient and more thermostable buffering by alkali-protein as the opportunity offers. That

it is this change over from one type of buffer to the other, which is the source of the delayed anaerobic heat, offers a simple explanation for this phenomenon. The complete change would produce a further 119 cals., so that, with only 74 cals. to account for, the reaction need not be assumed to proceed to completion, but only to the extent of about 60 p.c. The change is not likely to involve the lactic acid further, but to consist of a transfer of alkali from protein to acid phosphate and carbonic acid.

Hartree and Hill have further shown, that the velocity of the reaction responsible for the delayed anaerobic heat reaches a maximum about $2\frac{1}{2}$ minutes from its commencement, and is independent of the time of stimulus and temperature. If the reaction responsible for the delayed anaerobic heat were purely chemical in character, the independence of its velocity of time of stimulus and temperature would be difficult to explain; this difference is typical rather of a physical process. The difficulty, however, is easily overcome if it be imagined that the controlling factor in the velocity of reaction is the physical one of the rate of diffusion of the acid phosphate and carbonic acid ions through the muscle tissue, until they come in contact with an alkali-protein molecule.

Such a theory seems to demand some special distribution of phosphate and carbonate molecules in the muscle substance. We may imagine the small carbonic acid and phosphate molecules as carriers, going out from the neighbourhood of the immediate contractile mechanism, where they were initially situated, and reacting with the general muscle tissue by combining with alkali again, depositing their acid character, so to speak, on the heavy, less mobile, and less acid protein molecules. Such a reaction would reach a maximum velocity after a short period of time. The concentrated layer of molecules of acid salts existing immediately after relaxation would have at first no alkali protein within range with which to react, but as they diffused apart, each would have a greater opportunity of meeting the required type of protein molecule. The following scheme may make the hypothesis clearer:

Contraction. (*a*) Glycogen $\rightarrow$ lactic acid; (*b*) lactic acid and contractile mechanism produce mechanical response.

Relaxation. (*a*) Lactic acid $+ \left\{\begin{matrix} Na_2HPO_4 \\ NaHCO_3 \end{matrix}\right. \rightarrow$ Sodium lactate $+ \left\{\begin{matrix} NaH_2PO_4 \\ H_2CO_3 \end{matrix}\right.$.

Anaerobic recovery. (*a*) $\left\{\begin{matrix} NaH_2PO_4 + \text{Na protein} \\ H_2CO_3 \end{matrix}\right.$

$$\rightarrow \left\{\begin{matrix} Na_2HPO_4 + \text{H protein.} \\ NaHCO_3 \end{matrix}\right.$$

SUMMARY.

1. A redetermination has been made of the heat of combustion of glycogen in dilute solution, and the value, 3874 cals. per gm., obtained for the hydrated material $(C_6H_{10}O_5.H_2O)_n$. This value is about 100 cals. higher than that previously accepted.

2. It is possible, using this new figure, to explain the actual heat measurements on muscle entirely on chemical grounds, by assuming that the lactic acid formed from glycogen is first neutralised locally by alkaline salts, and that then more slowly these salts are restored at the expense of the alkali-protein of the general muscle tissue.

The author wishes to thank Prof. A. V. Hill, Mr A. D. Ritchie and Dr A. L. Robinson for the help and encouragement they have given during the progress of the work, and particularly to thank Dr Robinson for the suggestion to apply Atkins' method for the drying of the glycogen.

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LOCALISATION OF THE VASO-MOTOR CENTRE.

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It is now fifty years since Dittmar(1), by the method of successive section located a vaso-motor centre in the medulla, but except recently there has been, so far as we have been able to find, no systematic investigation of the degree to which the centre can be localised by stimulating the surface of the medulla. Laborde(2), using cats, found a point in the medulla where stimulation by piqûre caused slowing and even stopping of the heart *via* the vagus. The surface marking of this point was close to the inferior fovea, the actual centre being located in what is now known as the nucleus ambiguus. Miller and Bowman(3), by unipolar stimulation in the dog located the cardio-inhibitory centre "in the dorsal vagal nucleus (ala cinerea, trigonum vagi)." As they wished to observe only cardiac effects they took steps to annul any vaso-motor changes. Ranson and Billingsley(4), using cats, found in the floor of the ventricle two points which they called the pressor and depressor points. The pressor point was situated "at the apex of the ala cinerea or the fovea inferior"; the depressor point, stimulation of which caused a fall in blood-pressure even with vagi cut, was situated in the area postrema just lateral to the obex. It appears from comparing the two papers that the cardio-inhibitory centre of Miller and Bowman is identical in position with the depressor point of Ranson and Billingsley. The latter authors make, however, no mention in this connection of any cardio-inhibitory effects. "The same results," they write, "were obtained with the vagi cut as with them intact." They come to no conclusion as to whether their results are due to stimulation of afferent paths or whether they indicate the existence of a vaso-dilator as distinct from a vaso-constrictor centre. Bayliss(5) in discussing these experiments is inclined to the latter view. "These centres in the bulb," he says, "are, so far as present evidence goes, the supreme co-ordinating centres." Ranson and Billingsley are inclined to believe in the existence of a vaso-tonic as distinguished from a vaso-reflex centre. Their belief in this centre, which

they locate in the region of the facial colliculus, is based on what appears to us to be the very inadequate ground that stimulation of this region gave "somewhat inconstant pressor reactions." In postulating a vaso-tonic centre Ranson and Billingsley were influenced by the experiments of Porter(6) and of Porter and Turner(7). These workers had claimed to have identified a vaso-tonic as distinct from a vaso-reflex centre on the ground that it was possible by the injection of certain drugs such as curare and alcohol to intensify or abolish reflex vaso-motor reactions while leaving the vaso-motor tone unaltered. The other interpretations which can with equal reason be placed upon these observations have been discussed by Bayliss(5). Moreover it appears from the work of Porter and Turner that the existence of the vaso-tonic centre can only be revealed under very special circumstances. "It is easy," they write, "to obtain a lessening of the reflex, but the greatest care must be used if a perfect result is desired. The least mismanagement of the anæsthetic, or the least error in the amount of the alcohol, the spread of the injection or the interval between injection and stimulation will defeat the observation." It appears to us that if a new centre is to be established in all such empirical conditions there would seem to be no limit to the process.

The evidence given for the view that the vaso-motor mechanism in the medulla consists of separate centres is open to criticism, and from this point of view we have made experiments upon the effects of stimulating the medullary surface.

Method. Thirteen complete experiments were performed, all upon cats. They were previously anæsthetised, some with ether alone, some with C.E. mixture, while a preliminary injection of urethane (1 gm. per kilo.) or morphia (10 mgms. per kilo.) was sometimes given. We found that the form of the anæsthetic did not influence the result though the depth of narcosis had an effect to be described later. In some animals the blood-supply was temporarily diminished during the operation of exposing the medulla. Either the two common carotids were clamped or the two subclavians at a point proximal to the origin of the vertebrals. In such cases the arteries were released again before the medulla was stimulated. In a few experiments we clamped all four arteries but we soon gave this up since we formed the opinion that although the medulla continued to function as evidenced by normal respiration and blood-pressure it appeared to be less sensitive to subsequent stimulation.

The medulla was exposed in the following manner. A median incision was made over the occipital region and the occipital bone and atlas

vertebra cleared of their muscle attachments. The posterior occipito-atlantal ligament was then incised and removed. The projecting parts of the lambdoidal ridges were broken off by bone forceps and two sagittal saw-cuts made from these ridges to the foramen magnum, each cut being about 6 mm. from the mid-line. The part of the skull lying between these two cuts laterally and the lambdoidal ridges superiorly was removed with nibbling forceps. The hæmorrhage from the diploë which, of course, is always profuse was stopped by smearing the cut surface of bone with beeswax. In this way a hole was obtained of sufficient size to allow of the removal of the cerebellum by scooping it out piecemeal. This process left the floor of the fourth ventricle well exposed and easily accessible to stimulation. Except in the first experiment in which fine double electrodes were used, the medulla was stimulated by a unipolar stigmatic electrode, the indifferent electrode being stitched into the cheek. The shocks varied in strength from weak to moderately strong on the tongue. The blood-pressure record was taken from the right common carotid artery using an ordinary mercury manometer. In this manner we have thoroughly explored the floor of the ventricle.

Fall of blood-pressure. We have identified the "depressor point" of Ranson and Billingsley. Its usual position which is shown in the accompanying drawing is, as these authors describe, in the area postrema, just lateral to the obex. It is bilateral and generally extremely localised, being not more than 1 mm. in extent. Its position, however, is not constant for on three occasions we have found it as high up as the fovea inferior. The fall in blood-pressure which occurs when this point is stimulated after section of the vagi is shown in Fig. 2. The fall is evidently due to peripheral vaso-dilatation for the tracing shows no diminution in the output of the heart. With vagi intact there is in addition well-marked slowing of the heart (Fig. 3). The "depressor point" is therefore identical with the cardio-inhibitory point found by Laborde.

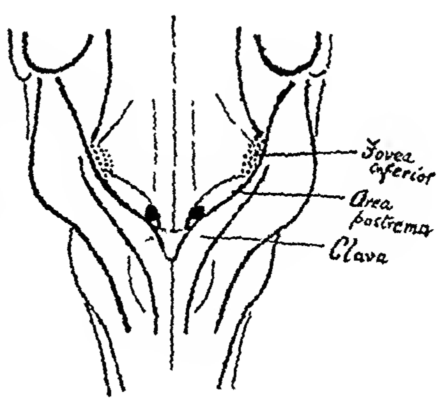

Fig. 1. Drawing of cat's medulla. × 3. The two large dots in the area postrema indicate the usual points, stimulation of which causes slowing of the heart and peripheral vaso-dilatation. The dotted area at the inferior fovea marks the region from which rise of pressure is most easily obtained.

When the vagus of one side is cut and the depressor point of the other side stimulated inhibition of the heart still occurs. This effect is not due

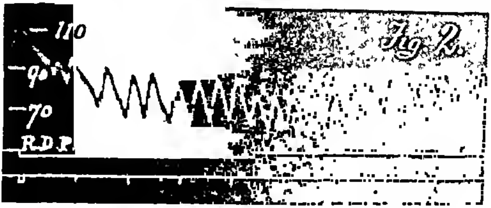

Fig. 2. Stimulation of depressor point, vagi cut.
Fig. 3. Stimulation of depressor point, vagi intact.

to spread of current as may be shown by the absence of cardio-inhibition when an equidistant neutral point is stimulated. There is evidently free decussation of fibres here. This region is the only one on the surface of the medulla stimulation of which produces both cardio-inhibition and peripheral vaso-dilatation.

The fact that stimulation of the one point produces both effects appears to us to point strongly to the fact that this is not a depressor point in the sense in which this expression is used by Ranson and Billingsley and by Bayliss, but rather that it represents a place where the afferent side of the depressor reflex arc comes near the surface, the part of the arc which is being stimulated being either the afferent fibres of the vagus or their terminations.

Other facts seem to support this view. First, there is a close similarity between the effects of stimulating this point and the effects of stimulating the central end of the vagus. Fig. 4 shows the resemblance in the slope of the blood-pressure fall in the two cases. Further, in both cases, the fall in pressure is usually accompanied by inhibition of respiration.

In those individuals in which the respiratory effect is slight in the one case it is slight also in the other. Secondly, our view is supported by anatomical evidence, for in the area postrema are nerve fibres in close association with the solitary bundle which is known to contain afferent vagus fibres.

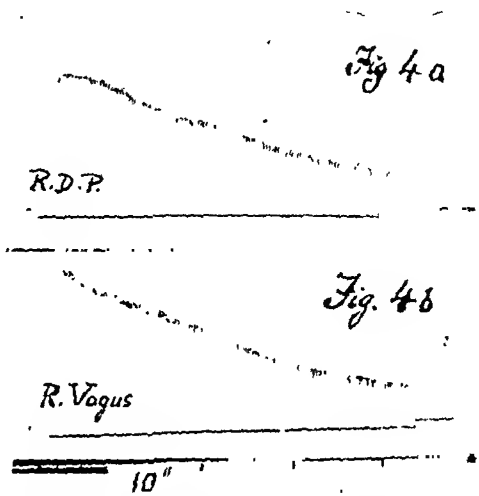

Fig. 4. Comparison of blood-pressure fall produced by stimulation of depressor point and by stimulation of the central end of the vagus.

Rise of blood-pressure. We have failed to identify any point which might be regarded as the pressor point. The point which Ranson and Billingsley found to give decided and constant pressor reactions we have found to give the most variable results. In four experiments we obtained a rise of blood-pressure accompanied in three of these by slowing of the heart. In six we obtained no decided effect while in the remaining three blood-pressure fell. We can only agree with Ranson and Billingsley to the extent that a pressor effect is more easily obtained from the fovea inferior than it is from any other point on the surface of the medulla. The best effect which we have succeeded in obtaining is shown in Fig. 5. Even here the rise in pressure is not so great as is commonly obtained from stimulation of any afferent nerve. Were the vaso-constrictor centre to be localised here a maximum rise should be constantly obtained.

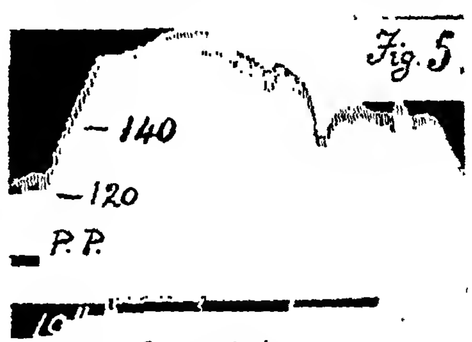

Fig. 5. Rise of pressure on stimulation of the fovea inferior.

The discrepancy between our results and those of Ranson and Billingsley we can only explain as due to difference either in the intensity of stimulation or in the depth of narcosis or a combination of both factors. Ranson and Billingsley admit that they were far from

obtaining pure pressor effects. "This response," they write, "may or may not be accompanied by respiratory inhibition, excessive respiration, cardio-inhibition, or slight movements of the head according to the stage of anæsthesia and other variable factors." We, too, have found some of these disturbances nearly always to accompany the rise of pressure. In only two instances could we obtain a purely pressor effect unaccompanied even by pricking of the ears. It is, moreover, noteworthy that in the figures published by Ranson and Billingsley, showing a well-marked rise of pressure the cat was under the influence of curare, a drug which would of course mask all motor manifestations.

The influence which the depth of anæsthesia and strength of stimulation exert is easily shown. It is possible in a lightly anæsthetised animal to obtain a pressor effect from almost any part of the medulla, even from the depressor point itself, provided that the current used is sufficiently strong.

The above facts lead us to believe that there is no single point on the surface of the medulla which can rightly be termed the pressor point. We hold that such pressor effects are to be more readily obtained by the assumption that we are stimulating unidentified nerve-cells and nerve-fibres, among them probably fibres of an afferent nature such as give a pressor effect when any afferent nerve is stimulated. Moreover, the experiments of Dittmar would not lead one to expect that blood-pressure is under the control of a centre so well localised as Ranson and Billingsley believe. For Dittmar found that on making serial sections the pressure fell gradually and he drew the conclusion that the centre was some 4 mm. in extent. In this he was confirmed by several workers subsequently.

Supposed vaso-tonic centre. We have noticed a dissociation of vaso-tonic from vaso-reflex effects for sometimes on ligature of the four arteries a high blood-pressure was maintained while the depressor reflex and the depressor effect on stimulating the depressor point were diminished or abolished. We believe, however, that for such a phenomenon several explanations are possible, as for instance Bayliss's hypothesis that the synapses are more readily paralysed by oxygen-want than are the nerve-cells. We can find no evidence for the existence of a vaso-tonic centre as distinguished from a vaso-reflex centre.

Conclusions.

1. We have confirmed the existence of a "depressor point" in the area postrema. It coincides with the so-called "cardio-inhibitory

centre." We believe it to represent a point where afferent vagal fibres belonging to the depressor arc occupy a superficial position.

2. There is free decussation of cardio-inhibitory fibres between the two depressor points.

3. The rise of pressure obtained sometimes from the fovea inferior is part of a mixed effect which for the present defies analysis. There is not sufficient evidence to localise a "vaso-constrictor centre" here.

4. Stimulation of the surface of the medulla gives no evidence of the existence of a vaso-tonic as distinct from a vaso-reflex centre, or of the existence of a vaso-dilator centre.

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A QUANTITATIVE COMPARISON BETWEEN THE ENERGY LIBERATED AND THE WORK PERFORMED BY THE ISOLATED SARTORIUS MUSCLE OF THE FROG. By WALLACE O. FENN.

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THIS work is the result of a suggestion by Prof. A. V. Hill that the heat production of muscles allowed to shorten and do work should be re-investigated by means of the improved myothermal methods which he and Hartree(1) have developed and have applied with such success to muscles in isometric contraction. As shown by their work on the difficult problem of the thermo-elastic effect(2), this technique makes it possible to allow muscles to shorten without errors due to slipping over the junctions provided strict precautions be taken to insure uniformity of temperature. The primary object of the experiments was to measure the maximum efficiency attainable with a frog's muscle under ideal conditions of load, etc. This has been done and the result shows an astonishingly low efficiency. The experiments, moreover, have led to important modifications of some of the fundamental and accepted principles of muscle physiology. In particular it can now be shown that there is a fairly good quantitative relation between the heat production of muscles and the work which they perform, and that a muscle which does work liberates, *ipso facto*, an extra supply of energy which does not appear in an isometric contraction. Having reached this rather novel point of view, it was a surprise to find that in its essentials it was not new but was urged by A. Fick 30 years ago, without, however, a satisfactory experimental basis. For this reason the history of the subject is of interest.

Heidenhain(3) first showed that if increasing weights were hung on a muscle (not after-loaded) then the heat production caused by the contraction of the muscle and the lifting of the weight increased with increase in the weight. Both Fick(4) and Heidenhain(3) added to this fact the observation that the heat also increased with the load even if the muscle always contracted from the same initial position. This was accomplished either by after-loading the muscle or by the use of Fick's

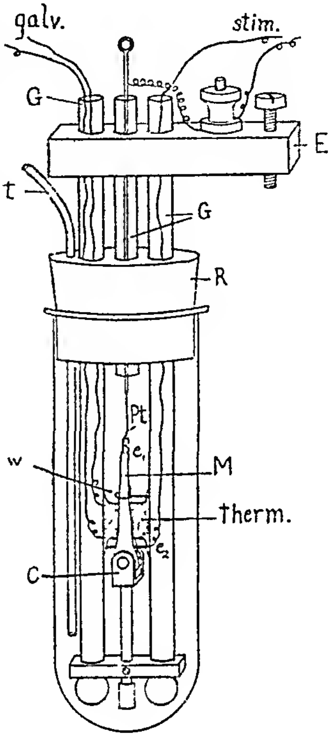
inertia lever. Fick, in consideration of the increase in heat which he obtained with increase in work, endeavoured to find out whether the mechanical potential energy set up during an isometric contraction could be used for mechanical work, or whether, when the muscle shortened and did work there was an extra equivalent amount of chemical energy liberated in the muscle. A complete answer to this question has not yet been found. It had been shown by Heidenhain(3) that a muscle gave off more heat in an isometric twitch than in a freely shortening twitch under varying loads, but this might mean merely that the process of shortening had prevented the development of the potential energy. Consequently Fick(5) arranged to hold the muscle in the isometric position until the maximum tension had been developed and then to release it to shorten "mit Wurf." Under these circumstances he found more heat in the muscle when allowed to shorten than when shortening was prevented altogether. He concluded that in muscle mechanical potential energy is not produced beforehand by chemical processes, being then available for doing mechanical work, but that in all probability the chemical process accompanying the mechanical effect occurs at the time when the mechanical effect is produced. This conclusion of Fick's might have been accepted, but Schenck(6) did not have much success in confirming his experiments except under special conditions. Greife(7) agreed with Heidenhain(3), Fick(6) and Schenck(6) that an isometric contraction gave in general more heat than an isotonic, but both Fick and Heidenhain found certain cases in which an isotonic contraction under a heavy load gave slightly more heat than an isometric contraction(9, 10). Blix(11), however, insisted that the isometric contraction always gave more heat than the isotonic, the reason being that the average length of the muscle was greater when the muscle was held fast than when shortening was permitted. In Fick's final paper on the subject(12) he arranged, with the use of Blix's myographion, first to let the muscle do work during stimulation and then to stretch it during stimulation. He found always less heat produced when work was done on the muscle than when the muscle did work. He concluded, therefore, "that it is the actual process of doing positive mechanical work, *i.e.* of shortening under tension, which is responsible, in muscle, for the chief expenditure of chemical energy." This conclusion, it now appears, was entirely correct, but it is an extremely difficult one to prove conclusively on a gastrocnemius or any similar muscle (cf. p. 196). Frank(13), in a review of the subject, therefore accepted Blix's hypothesis that variations in heat were due merely to variations in the length of the muscle.

Later A. V. Hill (14, 15), by improvements in the technique, made it appear that the earlier experiments were not very reliable and apparently settled the question once for all in favour of Blix by finding that the gastrocnemius and semimembranosus muscles gave off less heat in an isotonic contraction (without load) than in an isometric one, and that they gave off no more heat when shortening "mit Wurf" at the point of maximum tension (Fick's experiment) than when shortening was prevented altogether. Thus the view came to be accepted that on stimulation a muscle developed a given amount of heat and a given amount of elastic potential energy, both varying with the length of the fibres of the muscle. The amount of elastic potential energy which could be recovered as work depended merely upon the art of the experimenter in arranging his levers and had no relation to the total energy liberated. Tension was therefore regarded as the sole product of a muscular contraction.

Method. The method employed was that of Hartree and A. V. Hill (1). It will suffice here to indicate only certain modifications of their procedure. Most of the experiments were done with the large Paschen galvanometer supplied by the Cambridge and Paul Instrument Company, and purchased for the purpose by the generosity of the Rockefeller Institute for Medical Research, New York City, to whom grateful acknowledgment is made. For some of this work a combined thermopile and muscle chamber like that described by Hartree and Hill was used, but for the most part a somewhat simpler piece of apparatus of different design was employed. It is shown in Fig. 1. Its chief advantage is that, apart from the thermopile itself, it can be made very simply out of a rubber stopper, a test tube and some glass tubing. There is also less danger of leaks than with the ebonite box type and, due to the relative thinness of the glass walls, temperature equilibrium is attained more rapidly. The thermopile proper was made by electroplating silver upon constantan according to the method of W. H. Wilson (16) and was well insulated with shellac. The method of Hartree and Hill for calibrating the muscle had to be modified somewhat to suit a different set of apparatus. It consisted essentially in running an alternating current of known strength through the muscle for a known length of time. Current from the same source was used for stimulating the muscle where short tetani were wanted. The strength of the current was adjusted by means of a transformer and potentiometer and was measured by means of a special type of milliammeter in series with the muscle. The milliammeter consisted of a heating coil of very fine insulated constantan

wire (670 ohms) wound around the "hot" junctions of the thermopile which was used by Hill in his investigations on the heat associated with

Fig. 1. Thermopile and muscle chamber. Through the rubber stopper, *R*, of a large test tube pass three glass tubes, *G*. Between these the thermopile (therm.) is fastened by means of shellac and well insulated from the muscle by shellac. The leads from the thermopile to the galvanometer pass through two holes in one of the glass tubes. The lower stimulating electrode consists of a platinum bar, e_2, between the two muscles. It is connected to the stimulating circuit by means of a copper wire leading up through the other glass tube. The upper stimulating electrode, e_1, consists of a piece of platinum wire, *Pt*, bent into an 8-shaped loop at one end, which is tied between the two muscles and soldered to a fine steel rod at the other end. This rod serves to make mechanical connection with the lever and electrical connection with the electrode. *E* is an ebonite block by which the whole apparatus is secured upright in the water bath; *w* is a platinum wire guide for the muscle to insure good contact with the thermopile; *t* is a fine glass tube through which Ringer's solution or oxygen can be introduced into the chamber; *C* is an ebonite clamp to hold the muscle; it is secured to the glass rods by means of an ebonite bar at the base and a silver plated set screw. The whole apparatus is sunk well beneath the surface of the water bath which is stirred by a continuous current of air. The two glass tubes which conduct the wires to the surface are filled with paraffin to prevent leakage through the holes blown for the wires or through the external openings.



the transmission of the nervous impulse (17). The leads of this thermopile could be connected with the galvanometer and the deflection produced by a given current in the heating coil determined. A previous calibration of this milliammeter with a direct current of known strength and duration enabled the deflection produced by an alternating current to be read in absolute units if the sensitivity of the galvanometer were also determined. The resistance of the muscle was measured by substituting a known resistance for the muscle in the alternating heating circuit and varying it until the same deflection was obtained on the galvanometer, *i.e.* until the same current flowed, as when the muscle was used. In practice a measurement of the current was made with any known resistance and the resistance of the muscle could then be accurately calculated in obvious ways. The weight of the muscle between the electrodes was taken after each experiment.

Many of the phenomena described do not depend in any degree upon the accuracy of the calibration; they could be described equally well in

terms of the galvanometer deflection. In others, as will be seen, the accuracy of the result depends upon the determination of the heat in absolute units. Now the calibration procedure can be carried out nominally with any accuracy desired, but its significance always depends upon whether the artificial heating is exactly similar to the natural heating in distribution. It is exceedingly difficult to say just where musculature ends and tendon begins and to place an electrode on this exact spot in such a way that only the musculature will be heated by the current. It is equally difficult to correct for any error in the position of the electrode by measurements. Because of the difficulty of adjusting a ring electrode such as that used by Hartree and Hill for a muscle which must move freely through it, I have substituted a platinum vane tied between the ends of the two muscles (Fig. 1). Part of this vane certainly extends beyond the tendon to the musculature, but if it did not there would be an equal probability of error due to a warming of the tendon and to excessive production of heat in a poor contact between electrode and muscle. The accuracy of the calibration is also dependent upon the uniformity in the cross section of the muscle (which makes a gastrocnemius difficult to use) because the heat per unit length of muscle varies inversely as the cross section in an electrical calibration but directly as the cross section in a natural contraction. Attempts to control this error by measurements of the electrical resistance of different known lengths of the sartorius muscle have given rather variable results owing to the difficulties involved in measuring the short lengths accurately, but when averaged together they tend to show that the muscle is uniform in cross section, becoming correspondingly thinner where it becomes wider. This error must certainly be negligible in Hartree and Hill's experiments where both ends of the muscle can be neglected (see below). Another calibration error lies in the possibility of a shrinking or swelling of the muscle after killing with chloroform with a consequent change in its heat capacity and its position on the thermopile. Simple observation makes it appear probable that this error is usually negligible although the muscle occasionally shortens markedly under the influence of chloroform if not restrained.

The calibration is more difficult in these experiments where the total absolute amount of heat in the whole muscle must be known (in order to be compared with the total work done) than it is in Hartree and Hill's experiments where the force and heat developed *in the same length of muscle* are observed and where consequently any part of the muscle which projects beyond the electrodes at either end may be disregarded,

provided that the natural heat produced there is far enough away from the thermo-junctions to be unable of itself to affect the readings. The total error in the calibration in my experiments from all these causes cannot be accurately stated in figures, but it is believed that it could not by any possibility exceed 10 p.c. even in the small muscles; this is not serious for the purposes at hand. One specimen of *Rana esc.* was used. All the others were *R. temp.* or toads.

1. *Increase in total energy liberated with increase in work performed.*

The first experiments performed were a repetition of some made by Heidenhain and Fick. Any ordinary isotonic lever is used for recording the height of the contractions. A 25 or 50 gm. weight is hung on the lever so that the tension on the muscle is 5 to 10 gm. and its length only slightly exceeds its resting value. Any increase in the weight on the lever is supported by an after-loading screw. Short maximal tetani or maximal break shocks were used throughout for stimulating. In the contraction, energy which appears as mechanical work is stored temporarily in the lifted weight and reappears in the muscle as heat when the muscle relaxes and lowers the weight. The energy lost when the lever hits the after-loading screw has been calculated from the velocity of the weight at this moment, as measured from records of the contraction on a moving drum, and has been found to be negligible. Thus the galvanometer deflection represents the total energy mobilised as a result of the contraction. By means of the calibration this can be calculated in absolute units (ergs).

The protocol of a typical experiment is given in Table I in order to

TABLE I. Increase of heat with increase of work: typical exp.

Weight on lever gm.	Galvanometer deflection mm.		Height of contraction on drum cm.		Av. work Ergs × 10⁴	Av. heat Ergs × 10⁴	Efficiency W/H %
50	312	295	6·1	6·1	1·26	9·3	13·5
100	364	322	4·7	4·4	1·88	10·5	**17·9**
200	**412**	**338**	2·5	2·1	**1·90**	**11·5**	16·5
300	276	286	0·5	0·5	0·62	8·6	7·2
∞	238	↑	0	↑	0	7·3	0

Frog. Maximal alternating stimulus for 1/5 secs. At room temperature, viz. 7·5° C. Shortening = height on drum × 0·163. Work (ergs) = 981 × wt. on lever × height on drum × 0·0413 (lever factor). Heat (ergs) = mm. deflection × 306·2 (ergs per mm. from calibration). Two readings taken at each weight, first in ascending, then in descending order of weights as indicated by the arrows. Heat and work are expressed in units of 10,000 ergs. Weights in excess of 50 gm. were after-loaded. Maxima in heavy type.

make the procedure clear. The results of another experiment are plotted in Fig. 2. The important points to be noted are, (1) that less heat is

liberated in the isometric contraction than in any of those where shortening is allowed, (2) that the increase in heat (above the isometric heat) is roughly parallel to the work done, and (3) that the process in the muscle

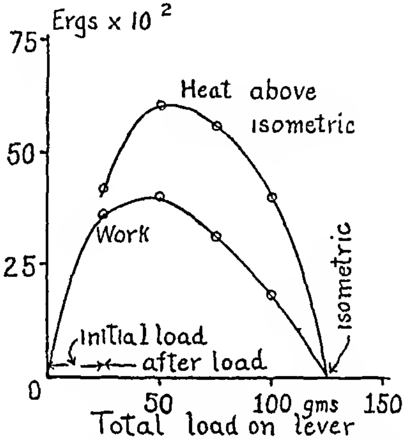


Fig. 2. Variations of work and heat (ordinates) in units of 100 ergs in isotonic contractions against increasing loads (abscissæ). The heat curve represents only the heat *in excess* of the isometric which was 193×10^2 ergs. Thus the isometric heat is made to coincide arbitrarily with the line of zero work. The muscle arm of the lever is 4·7 times the weight arm. Weight in excess of 25 gm. after-loaded. Muscle stimulated with maximal break shocks. Maximum efficiency of the initial process, $38{\cdot}7 - (193 + 61) = 15{\cdot}2\ \%$.

which causes this extra liberation of energy takes place after the stimulus is over (break shocks) and hence after the number of fibres brought into activity is presumably completely determined. This experiment supports the contention of Fick that work as well as tension is a product of the muscular reaction and it is much clearer evidence than can be obtained with the gastrocnemius muscle where the heat increases with increasing load but does not decrease again to the isometric.

Further data are given in Tables II and III. In some experiments the muscle was stimulated with a short tetanus; in others with a maximal break shock. The result is always the same, although the range of weights used may not always be sufficient to represent both sides of the maximum. In all, some 60 experiments of this type have been recorded. Typical variations of the heat with variations of work have

always been found except in a few cases when the muscle showed obvious signs of fatigue.

TABLE II. Increase of heat with increase of work.

(Summary of four exps. similar to that in Table I.)

Weight on lever gm.	Frog. 6·7° C.		Frog. 7·5° C.		Toad. 8° C.		Frog. 8·5° C.	
	Work	Heat	Work	Heat	Work	Heat	Work	Heat
50	1·30	10·1	1·46	14·9	0·26	1·45	0·58	5·03
100	2·01	13·1	2·62	17·1	0·24	1·37	0·74	5·64
200	1·56	14·4	3·26	16·4	0·02	1·14	0·42	5·50
300	0·18	9·6	1·79	14·5	—	—	0·12	4·82
∞	0	9·2	0	11·5	0	1·14	0	4·57
	0·5 sec.		0·5 sec.		0·2 sec.		Break shocks	

All exps. at room temperature, with a pair of sartorius muscles. Tension on muscle during contraction ¼ that of weight. Initial tension of muscle therefore 50/4 or 12·5 gm. Maxima in heavy type. Work and heat expressed in units of 10,000 ergs. Isotonic lever, after-loaded.

TABLE III. Increase of heat with increase of work.

(Summary of three exps. similar to that in Table I.)

	Toad. 8° C.		Frog. 0° C.		Frog. 8·5° C.	
25	4·3	24·1	3·5	23·4	3·1	43·8
50	5·1	20·7	3·9	25·4	4·1	38·5
75	4·3	18·3	3·2	24·9	3·9	37·4
100	2·4	14·4	1·8	23·2	3·0	35·0
150	0·2	13·1	0	19·3	0	29·6
∞	0	13·8	—		—	
	0·2 sec.		Break shocks		Break shocks	

Work and heat are averages of 2, 4 and 8 series respectively in alternating directions. Tension on muscle ¼ of weights given. Initial tension 6·25 gm. Maxima in heavy type. Units of 1000 ergs. Isotonic lever, after-loaded.

TABLE IV. Increase of heat with increase of work: constant shortening.

(Summary of six exps. similar to Fig. 3.)

	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
25	—	—	—	—	1·49	70·2	1·51	50·4
50	4·0	153	4·3	97	3·03	72·6	3·08	55·0
75	5·7	138	6·2	110	4·57	75·6	4·65	58·4
100	7·4	163	8·0	130	6·11	78·8	3·99	56·8
125	9·0	167	9·9	138	7·64	80·3	—	—
150	10·7	170	10·3	143	8·19	79·4	—	—
175	12·4	171	10·5	138	7·47	76·1	—	—
200	9·8	165	5·6	117	—	—	—	—
Isom. *l*	0	133	0	84	0	57·0	0	42·5
Isom. *s*	0	—	0	86	0	—	0	35·2
	0·5 sec., 8·4° C., 2·66 mm. shortening; muscle 0·19 gm.		0·4 sec., 0° C., 3·0 mm. shortening; muscle 0·25 gm.		0·2 sec., 8·2° C., 2·5 mm. shortening; muscle 0·26 gm.		0·2 sec., 0° C., 2·6 mm. shortening; muscle 0·18 gm.	

Frog. Tension on muscle during shortening ¼ that of weight given. The isometric heat in the long and the short positions is given where possible. Initial tension is represented by the smallest weight in each series. Each figure is the average of two series of ascending and descending weights respectively. When the work ceases to increase in proportion to the increase in weight, the shortening is incomplete. Maxima in heavy type. Units of 1000 ergs.

Blix (11) would explain the increase in heat with increase in load as due to an increased average length of fibre during the contraction. This

might have explained the initial rise but cannot account for the subsequent fall of the curve toward the isometric level: on the other hand the change in the heat is always proportional in a general way to the work. The work is equal to the product of the tension, T, under which the muscle shortens and the absolute amount of shortening, s As the load increases from 0 to infinity, Ts must pass through a maximum, since at zero weight $T = 0$ and at infinite weight (isometric) $s = 0$ With small loads the muscle shortens so much and so rapidly that maximum tension cannot be developed. With large loads the maximum tension is developed but the amount of shortening of which the muscle is capable before relaxation begins is very small.

2. *Increase in the total energy liberated when increasing loads are lifted to a constant height.*

In these experiments it is arranged that the muscle should shorten under different loads only between two fixed positions, the "long" position and the "short" position, the amount of shortening allowed being only 2 or 3 mm. A diagram of the apparatus designed for this purpose will be published later. In contraction the muscle lifts the weight from the long position to the short, and in relaxation the weight stretches the muscle again to the long position.

The purpose of this modification of the experiments described in the preceding section is to make possible a more accurate analysis of the factors of length, tension, shortening, etc , which together determine the amount of energy liberated in a muscle as a result of a given stimulus The advantages of the procedure are as follows

1. The amount of shortening remains constant over a considerable range of weights: hence variations in energy liberated must be due to variations in length of fibre, tension of the muscle during shortening, or speed of shortening.

2. Over this same range the maximum possible change of heat due to mere change in the length of the fibres can be determined by measuring the heat produced in an isometric contraction at each of these positions Thus, if the increase in heat due to an increase of load is much greater than the difference in the isometric heat at the long and short positions, then it is certain that the mere length of fibre is not the dominant factor

3. The change in shape of the muscle is also constant (until the weight becomes too heavy to lift to the required height) so that the irreversible "viscous" loss (cf. Hartree and Hill(2)) becomes proportional to the various speeds of shortening and lengthening, and hence its absolute

value for each weight can be estimated roughly by permitting the muscle to shorten from the long to the short position without being stimulated, then pulling it back immediately to the long position with a weight which is just adequate for the purpose, and measuring the resulting galvanometer deflection. Incidentally this procedure controls any large errors which might arise due to differences of temperature along the muscle. Usually one obtains a deflection of about 5 mm. which is perhaps 2 p.c. of the deflection obtained when the muscle contracts. This is so small that it has been neglected in recording the results, but if allowed for it would merely increase the amount of external work performed. This irreversible loss may of course be greater in a stimulated muscle than in a resting one, but no method has suggested itself of determining this point. When measured in this way on an unstimulated muscle the speed of shortening is somewhat less than in a contraction under the smallest weights, but the speed of lengthening is greater than in a stimulated muscle. A correction for the speed of the change of shape therefore would not significantly alter the above estimate that the irreversible "viscous" loss amounts to less than 5 p.c. of the total energy liberated.

4. This procedure also certifies that if the weight lifted is so small that the muscle cannot develop its maximum tension during the shortening, that tension can still be developed when the contraction becomes isometric at its shorter length.

5. When the muscle is *in situ* in the body of the frog it also contracts between fixed limits which are in a rough way comparable to those used experimentally.

In measuring the isometric heat in the "long" and "short" positions the muscle must also be calibrated in both positions. The calibration constants so obtained show that on the average 1·05 times as much heat must be liberated in the long position as in the short position in order to produce the same galvanometer deflection. This is because a stretched muscle is thinner on the thermopile. The "long" calibration is of course used for all readings where work is done, because the muscle is in that position throughout the duration of the galvanometer deflection (4 secs.) except for the first second or less.

The results of a typical experiment with limited shortening are plotted in Fig. 3. In general the curves may be regarded as comparable to those in Fig. 2 except that the ascending limb of both the heat and the work curves has been "sliced" off by the arbitrary limitation of the amount of shortening permitted. In Fig. 3, however, the base line of the work curve (work = 0) is plotted so as to coincide with the point

on the axis of ordinates representing the heat produced by shortening under zero load. In Fig. 2, on the other hand, the level of zero work

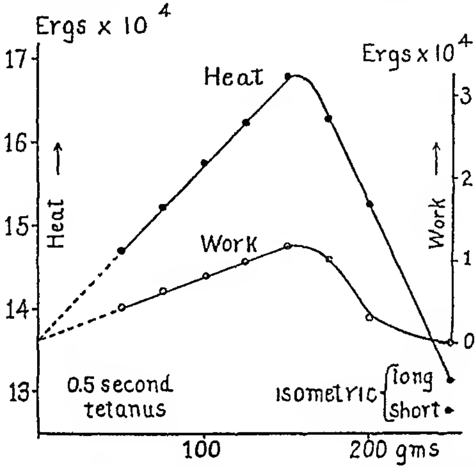


Fig 3 Exp similar to that in Fig 2 except that the amount of shortening was limited to 2 06 mm The heat curve has been extrapolated to the ordinate of zero load and the base line of the work curve is made to coincide arbitrarily with this point The work curve falls off at the point where the load becomes too heavy to be lifted the full distance Weights in excess of 50 gm after loaded Muscle arm on lever four times weight arm Weight of muscle between electrodes 0 19 gm Temp =8 4° C Results of four other similar experiments given in Table IV.

coincides with the level of isometric heat. This change is made in Fig. 3 in order that the ratio between the slopes of the heat and the work curves can be seen at a glance. In this experiment the ratio is 2·7. In 32 similar curves which have been obtained the average of this ratio was 2·3, *i.e.* the increase of energy liberated was 2·3 times as great as the increase in work In this average, two or three experiments have been neglected where the ratio was practically zero, due very evidently to the extreme fatigue of the muscle as shown by a very low and rapidly falling heat output and work output. Data from four normal experiments are summarised in Table IV (*supra*) In an experiment like that in Fig 3 it is impossible to start with zero weight because the muscle must be made to return to its original length promptly. The curves have

been prolonged, however (dotted), to the vertical axis. Even at this extrapolated point there is usually more energy liberated than in an isometric contraction at either the long or the short position, but the difference is not large. As the weight lifted is increased the work must increase in direct proportion, *i.e.* as a straight line, as long as the height to which the weight is lifted remains constant. When the weight becomes too heavy for the muscle to lift the required height the work curve falls off and finally decreases to 0 when the contraction becomes isometric. Usually, as in Fig. 3, the heat also increases approximately as a straight line until the maximum is reached and then decreases rapidly to the isometric level. Frequently the heat curve maximum occurs at a slightly lower weight than the work curve maximum. Examples of this may be found in Tables II, III and IV. This is probably to be explained by minor complications due to length of fibre and "viscous" loss. The mechanical efficiency (work divided by total energy liberated) disregarding the recovery heat production, is seldom over 10 p.c. in these experiments on account of the arbitrary limitation of the amount of shortening. The efficiency can readily be calculated from Table IV.

It was found by Evans and Hill(18) that the heat produced by the sartorius muscle in isometric contractions was greatest when the length of the muscle was approximately equal to its length in the frog. I have repeated their experiments with several muscles stimulated with break shocks and short tetani and can entirely confirm their conclusion. Moreover, I have had occasion to measure the isometric heat production in 14 experiments in the long and short positions, representing a change in length of 2½ to 3 mm. or about 10 p.c. of the length of the muscle. In five of these the heat production was 5 p.c. more in the long position than in the short; in nine it was 11 p.c. less. The initial tension was always between 6 and 12 gm. which is not far from the tension of the muscle in the body when the leg is extended. Thus in four muscles I have found that an initial tension of 9, 12, 19 and 25 gm. stretched the muscle to 102, 100, 106 and 110 p.c. respectively of their several normal extended lengths in the frog. Accurate measurements of muscle lengths are admittedly difficult to make, but it seems not unfair to say from these facts that in my experiments the muscles have worked over a range of lengths which is approximately normal. Any deviation from the normal length of the muscle diminishes the heat liberated isometrically on a given stimulus. It is perhaps worth emphasising that the proportionality between isometric heat and muscle length, or the area of certain longitudinal surfaces, which is so often quoted as an argument in favour of the

surface tension theory of muscular contraction, is true only over a very limited range.

Summary. 1. When a muscle is made to lift increasing weights through a constant small distance, comparable to the normal amount of shortening in the frog, the increase in energy liberated tends to be on the average 2·3 times the increase in work, *i.e.* it is proportional to the tension under which the shortening takes place. Complications due to fibre length and irreversible "viscous" loss can be largely ruled out.

2. When a muscle shortens 2 or 3 mm. under some tension the energy liberated is greater than the time average of the isometric heat for all the lengths through which the muscle passes in shortening. When there is no weight on the muscle this is usually but not always true.

3. *Increase in total energy liberated caused by increased degree of shortening under a constant load.*

It has been shown above that the heat production of muscles increases when increasing loads are lifted through the same distance. Evidence will now be presented that the converse is also true, *i.e.* that the heat production of muscles is increased when the same load is lifted through increasing distances.

The amount of shortening was varied by adjusting the proper screw. Each series started with no shortening, *i.e.* isometric. The shortening was then increased at random until the weight could be lifted no higher, after which the shortening was decreased again step by step to the isometric. The results obtained with 100, 50 and 25 gm. on the lever using the same muscle are plotted in Fig. 4. The curves H_{100}, H_{50} and H_{25} show the increase in heat above the isometric with the three different weights; the broken lines W_{100}, W_{50} and W_{25} represent the corresponding increase in work. There is a tendency for the curves to reach a maximum, but it is obviously a fair approximation to the truth to say that, with a given weight, the increase in heat is proportional to the shortening. Furthermore, the rate of increase of heat with increased shortening is roughly proportional to the tension under which the muscle shortens. As far as the muscle is concerned, the two factors which determine the amount of work performed are the amount of shortening and the tension during shortening. This experiment, of itself, proves that it is neither one of these factors alone but rather their

averaged. One series starts with an infinite equivalent mass (isometric) and ends with the smallest equivalent mass, while the other series is similar but in reverse order. The results of such an experiment are shown in Fig. 5 where the work, and the extra heat liberated because of this

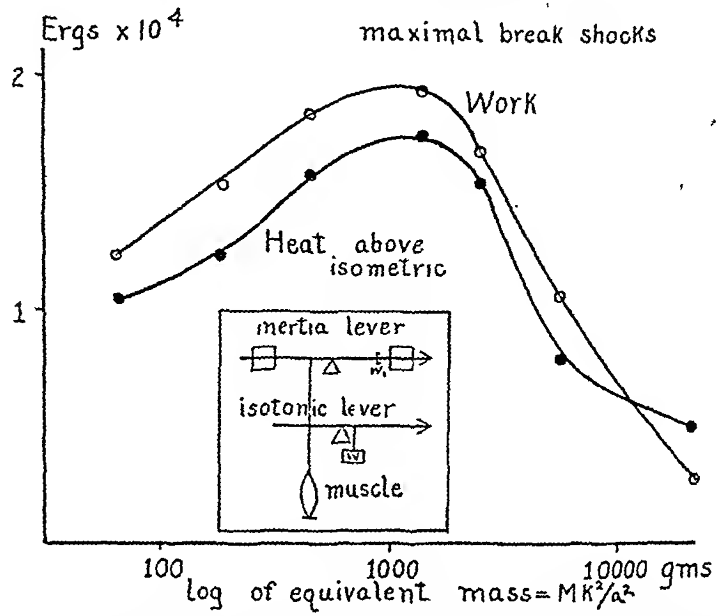


Fig. 5. Energy liberated in excess of isometric, and the work performed when a muscle is stimulated with maximal break shocks and made to shorten against an inertia lever. "Equivalent mass" of lever increased by moving point of attachment of muscle farther away from fulcrum. "Equivalent masses" are represented as abscissæ; logarithms have been used merely for convenience in plotting. At large equivalent masses the work done on the isotonic lever is practically negligible, but at small equivalent masses it becomes a large fraction of the whole. Isometric heat 97,300 ergs. Room temp. = 8·5° C. Weight of muscle between electrodes 0·26 gm. Each point is the average of six determinations on the same muscle.

work (heat above isometric), both in units of 10,000 ergs, are plotted as ordinates over the logarithms of the equivalent masses as abscissæ. The nearer the point of attachment of the muscle to the fulcrum, the greater the equivalent mass, the less the shortening of the muscle, the slower the shortening, and the greater the tension on the muscle. In this experiment, within the limits of error of the calibration, the extra heat liberated is equal to the work done. This is frequently, but by no means always, the case. This particular experiment was selected for plotting because break shocks were used for stimulating, thus proving that the excess heat due to the work done may be liberated after the

stimulus is over and hence cannot be due to an increased number of fibres brought into contraction.

Averaging the results of 33 experiments like that recorded in Fig. 5 it is found that the extra heat is 1·3 times as great as the work performed at the optimum equivalent mass. Averaging similarly 36 experiments like that in Fig. 2 in which increasing weights on an *isotonic* lever were lifted by a muscle it was found that the extra heat liberated was 1·8 times as great as the work done with the optimum weight. The difference between 1·8 on the isotonic lever and 1·3 on the inertia lever is significant and is due largely to the fact that in the experiment with the isotonic lever the muscle had to let the weight down again in relaxation. It will be shown in another paper that there is an extra expenditure of energy involved in lowering a weight in relaxation which will account for the difference mentioned. A small part of the difference might be due to the fact that the maximum mechanical efficiency of a contraction on the inertia lever is 20 p.c. greater than the maximum on the isotonic lever, *i.e.* there is more work done on the inertia lever for the same amount of heat. The maximum energy liberated on the isotonic lever tends to be greater, however, than on the inertia lever, showing definitely that some other factor such as that mentioned must be involved.

In Table V the results of seven other experiments similar to that in

TABLE V. Increase of heat with increase of work on inertia lever. (Summary of seven exps.)

Exps. 1, 2 and 3			Exps. 4 and 5			Exps. 6 and 7		
Equiv. mass (kilos.)	Work	Excess heat	Equiv. mass (kilos.)	Work	Excess heat	Equiv. mass (kilos.)	Work	Excess heat
0·32	62	111	0·78	68	122	0·36	217	276
0·40	64	111	1·12	60	117	0·80	264	420
0·72	65	108	1·75	70	114	1·42	288	493
1·29	66	97	3·13	69	107	3·10	308	508
1·85	63	86	4·48	67	100	5·67	281	460
2·89	59	85	7·01	64	88	12·76	236	352
5·15	49	59	12·40	53	64	—	—	—
11·58	28	33	28·00	40	41	—	—	—
46·34	12	16	112·2	18	12	—	—	—
Isom. 33,460 ergs, 0·5 sec. Av. of 8 dets. on 3 muscles at 7° C.			Isom. 28,800 ergs, 0·4 and 0·5 sec. Av. of 6 dets. on 2 muscles at 0° C.			Isom. 103,500 ergs, 0·5 sec. Av. of 4 dets. on 2 muscles at 7° C.		

Excess heat is total heat minus isometric heat. Work and heat in units of 100 ergs. All at room temperature unless at 0° C. Similar experiments only have been averaged together.

Fig. 5 are summarised. Similar experiments have been averaged together. In all but the last two the heat is at a maximum at the smallest

equivalent mass used, where the work is slightly below the maximum. The same thing can be observed in experiments on the isotonic lever as shown in Tables II, III and IV, where the maximum heat production frequently occurs with a slightly smaller load than the maximum work. In Exps. 6 and 7 in Table V the muscles used were stronger than in the other experiments. Their average maximum work is nearly five times as large and the optimum equivalent mass is also larger.

In one experiment with the inertia lever at 6·7° C. I have been able to show that an increase in the duration of the stimulus from 0·05 to 0·7 sec. increased the optimum equivalent mass from 40 or 80 to 5670 gm., although the maximum efficiency was just 20 p.c. in both cases. In another similar experiment at 0° C., an increase in the duration of the stimulus from 0·05 to 0·5 sec. increased the optimum equivalent mass from 1400 to 3150 gm. and increased the maximum efficiency slightly from 14 p.c. to 15·5 p.c. The experiments were conducted by using first one duration of stimulus and then the other in alternation in order to eliminate changes due to fatigue.

The interpretation of the curves in Fig. 5 is as follows. The work performed by a muscle in any contraction is equal to the product of the average tension of the muscle by the amount of shortening. As the equivalent mass is decreased the amount of shortening increases and becomes so rapid that the tension of the muscle decreases and approaches zero. Conversely as the equivalent mass is increased the tension increases, but the amount of shortening which can be accomplished before the stimulus is over and relaxation begins decreases and approaches zero (isometric). Hence the product of shortening and tension, *i.e.* work, must pass through a maximum at a certain optimum equivalent mass, for this product is necessarily equal to zero at infinitely large or infinitely small equivalent masses. If the stimulus were continued until a given amount of shortening were accomplished, as in Hill's experiments with human arm muscles pulling against an inertia device(22), there would be no optimum equivalent mass but the work would continue to increase indefinitely with the mass. It will be important to find out to what extent the theoretical interpretation of Hill's curves will require modification in the light of the knowledge that the shortening of a stimulated muscle is not comparable to the shortening of a stretched spring but is more like the behaviour of an electric motor. If the muscle were like a spring it would possess a given amount of potential energy which, if it did not appear as work, would necessarily appear as heat in some irreversible "viscous" process involved in the change of shape. If, however, the muscle were like an electric motor it would develop less energy where the equivalent mass was small and it would be unnecessary to postulate a large frictional loss. It remains for future investigation, however,

to prove the applicability of these experiments on frogs to human muscles.

Summary. 1. When a muscle shortens against an inertia lever it liberates more energy than in an isometric contraction. This excess energy liberated is on the average 1·3 times as great as the work performed.

2. For a given duration of stimulus there is an optimum equivalent mass at which the maximum work is done with maximum efficiency. The optimum equivalent mass is greater for a long stimulus than for a short one.

5. *The mechanical efficiency of isolated sartorius muscles.*

The direct measurement of the mechanical anaerobic efficiency of isolated muscles has been made by Meyerhof (21), using a determination of the amount of lactic acid produced by the muscle as a measure of the total energy liberated. He arrived at an estimate of 45–50 p.c. after endeavouring to allow for the fatigue which was an inevitable part of his method due to the necessity of stimulating the muscles many times in order to develop enough lactic acid for an accurate measurement. Fick ((9), p. 221) found efficiencies of about 25 p.c. by the thermo-electric method, without however a satisfactory method of calibrating the muscle and so of translating the galvanometer deflection into absolute units.

It had been supposed for two reasons that the efficiency might be as high as 75 p.c. if the energy used in oxidative recovery was disregarded, *i.e.* if the energy expenditure was calculated from the initial heat production as given by the galvanometer deflection. The reasons for this prediction were, firstly, that oxidative efficiencies have been recorded in man as high as 30 p.c. Assuming, with A. V. Hill (23), that the oxidative heat production is 1·5 times the initial heat production, the total heat would be 2·5 times the initial heat and the anaerobic efficiency would be 2·5 × 30 p.c. or 75 p.c. The second reason originated from a consideration of the theoretical maximum work of a frog's muscle as measured by the area under the curve obtained by plotting the maximum isometric tension of the muscle for a given stimulus against the corresponding length of the muscle. This theoretical maximum work was found by A. V. Hill (14) to be a large fraction of the initial heat production. If then the muscle could be so loaded that it could attain the maximum tension at every stage in its shortening, when stimulated, then the mechanical work done would be equal to that calculated from the length-tension diagram and the efficiency would be high. It was hoped that an approximation to an ideal condition of loading could be obtained with the inertia lever.

The actual results obtained did not verify the predictions (see Table VI). Each figure there given is the maximum efficiency obtained with one of

TABLE VI. Mechanical efficiency of isolated muscles working on isotonic and inertia levers.

Frog.	Isotonic:	28, 26, 25, 23, 21, 19, 19, 17, 16, 14, 13. Av. 20 %.
	Inertia:	32, 27, 27, 26, 25, 25, 23, 23, 23, 23, 22, 22, 21, 21, 20, 20, 16. Av. 23 %.
Toad.	Isotonic:	25, 22, 19, 18. Av. 21 %.
	Inertia:	40, 36, 31, 31, 25, 22, 17. Av. 29 %.

Each figure represents the highest value which could be obtained from one muscle in a large number of trials.

the pairs of muscles used. The measurements covered a period from August to May and the temperature varied from 0° to 15° C. The differences due to temperature were not larger than the differences due to varying conditions of the muscles so that no temperature effect can be observed. The muscles worked on both an isotonic and an inertia lever. The results are arranged in the order of magnitude so that the first figure represents the maximum. Muscles of toads appear to be somewhat more efficient than those of frogs. This may be correlated with the slower movements of the former, the frog being built for speed rather than efficiency. The efficiencies recorded on the inertia lever are somewhat higher than those on the isotonic lever. An average of the efficiencies obtained when the same muscle was used on both kinds of levers shows that the isotonic lever gives efficiencies only 79 p.c. as high as the inertia lever.

On the whole the efficiency of an isolated sartorius muscle of the frog may be taken to be between 25 and 30 p.c. This means an *oxidative* efficiency only two-fifths as great, *i.e.* 10 to 12 p.c., which is only one-half or one-third as great as the oxidative efficiencies usually recorded on man. Possibly the frog is to be regarded as a very inefficient animal compared to man, being built for quick movements at low temperatures where man's muscles would move more slowly and waste less energy in change of shape. Possibly also abnormalities caused by the removal of the muscle from the body may have something to do with the low efficiencies obtained.

The reasons why the predictions of a high efficiency were not realised are twofold:

1. As shown by A. V. Hill(24) and Meyerhof(21) the theoretical maximum work cannot be attained in practice in a single contraction unless sufficient time is allowed at each length for the maximum tension to be developed. Even on the inertia lever Doi(20) found that this condition does not obtain. Hill states that the theoretical maximum work

equals $1/6\ Tl$, T being the maximum isometric tension and l the length of the muscle. Doi found that the maximum work performed was only $1/25$ or $1/20\ Tl$.

2. Hill's predictions of a high efficiency were based upon the hitherto commonly accepted assumption that the energy liberated depends entirely upon the nature of the stimulus and the initial condition of the muscle, and is independent of the weight lifted. If this had been so the experiments would have justified his use of the graph in which isometric tension is plotted against length for predicting the behaviour of a muscle when allowed to shorten. Thus, if the efficiencies recorded in Table VI be recalculated, substituting the isometric heat for the total energy actually liberated at the point of maximum efficiency, much higher efficiencies are obtained. This can be done roughly on the assumption that the excess heat liberated is 1·3 times the work performed on the inertia lever. Now the highest efficiency recorded in Table VI is 40 p.c.[1] for a toad. The total energy may be taken as 100, the work as 40, and the excess heat above the isometric as $1{\cdot}3 \times 40$ or 52. The isometric heat would then be $100 - 52$ or 48 and the efficiency, if heat had been independent of work, would have been 40/48 or 83 p.c. Similarly taking 32 p.c., the highest individual record for a frog, it can be calculated that the isometric heat would have been $100 - (1{\cdot}3 \times 32)$ or 58, and the efficiency would have been 32/58 or 55 p.c.

But while these considerations justify the predictions on the evidence then available they raise another more fundamental question, for the existence of this excess energy liberation appears to be incompatible with the assumption that a stimulated muscle is a new elastic body possessing elastic potential energy. The shortening of a muscle appears to be an active process and not merely analogous to the release of a spring previously stretched. The energy used in the performance of work is developed at the time when the work is done and does not represent potential energy already developed before shortening begins. Hence estimates of the work which a muscle can do cannot logically be based upon evidence obtained from isometric contractions.

Summary. The *oxidative* efficiency of an isolated frog's sartorius muscle is only 10 to 12 p.c.

[1] This figure is to be accepted with some caution because it has never been repeated on another muscle. This animal, however, was the largest and most healthy toad which I had an opportunity of using and it repeatedly gave efficiencies of this order of magnitude.

6. *Comparison between gastrocnemius and sartorius muscles.*

All the earlier work on the heat production of muscles allowed to shorten and do work was done on the gastrocnemius or semimembranosus muscles or the preparation described by Fick((9), p. 11), consisting of the inner side of the thigh joining the pelvis and the knee. All the differences between my results on the sartorius muscle and earlier ones can be reduced to the fact that the isometric heat has previously been found too high relative to the isotonic heat. It seems probable that this difference is an anatomical one and is due to the fact that an "isometric" contraction of a non-parallel-fibred muscle like the gastrocnemius is not isometric at all as far as the individual fibres are concerned. When such a muscle is stimulated with the two ends held fast there appears to be a change in shape which necessarily implies a shortening of some fibres with a consequent stretching of other fibres or of the tendon. The isometric contraction of the gastrocnemius muscle is, therefore, a contraction in which there is a slight shortening of the individual muscle fibres under maximal tension. Taking the thin parallel-fibred sartorius muscle as the best available example of a single muscle fibre and admitting my experiments on this muscle as evidence of its behaviour, it becomes clear why the isometric contraction of the gastrocnemius should liberate relatively too much heat, for in the sartorius even a very slight shortening if carried out under maximal tension causes a considerable excess heat liberation. It can be calculated, for example, from the data in Table I that a shortening of 0·8 mm., or about 3 p.c. (0·5 cm. on the drum), under a tension of 300 gm. (on the lever) increased the heat production 18 p.c. above the isometric. Similarly Hartree and Hill, in their experiments (unpublished) on the sartorius muscle, have found that a muscle tied "isometrically" with a thread gives off notably more heat than one similarly stimulated but tied with an inextensible wire. The thread stretches enough to enable the muscle to do an appreciable amount of work and hence to liberate a corresponding extra amount of heat. For these reasons the anatomical structure of a muscle, the arrangement of its fibres, the elasticity of its tendon, etc., has much to do with the relative amounts of isotonic and isometric heat.

There must be few, if any, muscular contractions in the living body which can really be carried out isometrically; there is always some bulging or twisting of the muscle which implies more or less shortening of the fibres. It is probable that even in a sartorius muscle the contraction

is not really isometric unless the muscle is so stimulated that all the fibres are brought into activity simultaneously. Thus Bethe(25) has recently shown by a photographic method that in a muscle stimulated through its nerve a wave of contraction passes over the muscles in such a way that fibres remote from the origin of the wave are stretched before the wave reaches them by the contraction of fibres nearer the origin. In a muscle stimulated, as in my experiments, by an alternating current running from one end of the muscle to the other this initial stretching fails to occur. It will be interesting to confirm this view by measurements of the isometric heat of sartorius muscles stimulated in various ways with maximal stimuli of constant duration.

Prof. Hill has suggested to me that these same considerations may explain the observations of Hartree and Hill(26) that the isometric heat frequently decreases though the maximum tension remains the same, when the strength of the stimulating current is increased above the maximal. With a current just maximal some fibres might be stimulated slightly sooner than others, perhaps due to a better supply of nerve fibres, and would thus have an opportunity of shortening slightly in contraction. A still stronger stimulus might well prevent this shortening by bringing all the fibres into activity simultaneously.

In order to make certain that my results do not differ from the older results merely because of some modification in the method, I have repeated some of my experiments using a pair of gastrocnemius muscles in place of the sartorius and I have had no difficulty in confirming the older work in the following essential points.

1. As the after-load is increased the heat production continues to increase until the maximum is reached at infinite load, *i.e.* isometric. This I suggest is due to the fact that there is considerable shortening of the fibres even in an isometric contraction. In the few experiments which I have tried I have not been able to confirm the observation of Heidenhain and Fick(20) that the heat passes sometimes through a slight maximum just before the isometric is reached, thus suggesting the type of curve which I have found invariably on the sartorius. If their observations are trusted, however, in spite of Blix's emphatic denial of them, they may be taken as evidence that the increase of heat with increase of work is a factor in the gastrocnemius curves even though the factor of fibre length may also be important.

2. With a single gastrocnemius muscle I have repeated and confirmed Hill's experiment(14) in which the muscle was prevented from shortening for increasing times after stimulation with a consequent increase in the

heat production. In my experiment, as in Fick's(5), there was evidence of a maximum in the heat production just before the contraction became isometric (at point of maximum tension). Schenck(6) found this maximum only under special conditions when neither the tension nor the shortening was too small. This fits in perfectly with the idea that the isotonic heat is at a maximum under conditions which make the work maximal also and it doubtless accounts for Hill's result with an unloaded muscle in which the isometric contraction gave the maximum heat.

Hill(14) has shown that the theoretical maximum work (the area of the tension-length diagram) of a sartorius muscle is 1/6 the product of the isometric tension T, at the greatest length, and the length of the muscle l. In the gastrocnemius, Meyerhof(21) has found that the theoretical maximum work is only 1/14 or 1/17 of Tl. Hill suggests (unpublished) that this difference is also a matter of anatomy. T is the sum—or the resultant—of the tension of the individual fibres of the muscle; l the average length of one of them. If the length of the fibres of the gastrocnemius is only 6/14 or 6/17 that of the muscle while the length of the sartorius fibres is equal to that of the whole muscle, the difference is completely resolved.

It is already well known that the sartorius with its straight fibres is adapted for long quick movements under light loads, while the gastrocnemius with its diagonal fibres is adapted for the development of high tension with very little shortening. It seems possible that the gastrocnemius would be assisted in developing a high tension by the slight shortening of the fibres which always takes place in its supposedly isometric contraction and the consequent liberation of an extra supply of energy. Thus Hill(14) has observed that a 35 p.c. greater tension can be developed by a stimulated muscle at a given length if it has had to do work before reaching that length. The peculiar significance of this fact for the gastrocnemius and similar muscles now becomes apparent.

From this discussion it appears likely that the results which have een described for a sart[illegible]us muscle are equally applicable to other types

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muscle, I would claim for my experiments

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[illegible]re of the contractile process.

[illegible]plication to the heart. It is a familiar fact(27,28) that the oxygen

consumption increases with increase in the work of the heart, whether it is the pressure or the output which is varied. Similarly, it can now be shown that the energy liberated by the frog's skeletal muscle varies with the work, whether it is the load or the shortening which is increased. I have shown that this phenomenon in skeletal muscle *cannot* be explained by changes in the length of the muscle fibres and that the significant factors are rather the amount of shortening of the fibres and the tension under which this shortening takes place. It is possible, indeed probable, that these same two factors (the product of which is work) determine the energy liberated and hence the oxygen consumption of the heart. The advantage to the heart of a greater initial volume may thus be due not to a greater length of fibre *per se* but rather to more nearly optimal mechanical conditions of working caused by an increased tension of the fibres during shortening (for the same arterial pressure) and a decreased amount of shortening (for the same volume output).

It is impossible at present to evaluate the true effect of fibre length on the energy liberated "isometrically" on account of the inevitable shortening which must occur, particularly in the heart. Even if fibre length had *no* effect on the energy liberated, an increase in the length of the whole muscle (in the gastrocnemius and heart at least) might be expected to cause the energy to pass through a maximum, as it is observed to do, for in stretching the muscle, the "isometric" shortening of the fibres approaches zero while the tension during that shortening approaches infinity. *All* the effect of fibre length on energy output cannot be explained in this way unless there is likewise an "isometric" shortening of fibres in the sartorius sufficient to explain the same phenomenon in that muscle.

The all-or-none law.

If a muscle *fibre* contracts at all as a result of a stimulus, the tension developed, the work done, and the energy liberated by that contraction will be (1) independent of the strength of the stimulus, and (2) dependent only upon the initial physiological and mechanical conditions which obtain in the muscle. This I take to be the commonly accepted statement of the all-or-none law as applied to muscles. Hartree and Hill(26) brought forward an apparent exception to item (1) when they observed that the heat production of a muscle is frequently slightly less with supermaximal stimuli than with maximal stimuli, showing that the contraction is not quite independent of the stimulus. This cannot now be regarded as necessarily an exception for a possible alternative explanation of the observation has been suggested above.

My experiments show, however, that item (2) in the above statement of the law is emphatically incorrect for the total energy can be varied markedly by changes in the tension and length of the muscle which occur *after* the stimulus is over. The energy of the contraction is dependent not only upon the initial conditions of the muscle but also upon the conditions which obtain during and for some time after the stimulus.

It is to some extent a corollary of the all-or-none law that variations in the energy output of a whole muscle for a given duration of contraction are due to variations in the number of active fibres. It now appears, however, that there is another regulatory mechanism within each individual fibre which, within certain limits, is able to adapt the energy output to the work done. When we lift a weight, therefore, two regulatory mechanisms are in operation. One is dependent upon the nervous system and acts by stimulating more and more fibres according to need. It can therefore vary the total energy liberated by small *steps* over a wide range of values, each step representing the contribution of a single fibre. The other mechanism is independent of the nervous system and works merely by virtue of the fundamental nature of the muscle machine, whatever that may be. By its means the energy output of each individual fibre can be promptly and more or less precisely adjusted to its work. This second mechanism may be regarded as a sort of fine adjustment, instantaneously active but capable of producing only relatively small variations in energy output.

Summary. 1. The all-or-none law, as applied to muscles, must be confined to the statement that the total energy liberated in a contraction of a single muscle fibre is independent of the strength of the stimulus.

2. The energy expenditure of a single muscle fibre can be varied by changes in the mechanical conditions of the muscle which occur *after* the stimulus is over.

The general nature of the contractile mechanism.

It is hoped that in the preceding pages convincing evidence has been presented to show that the initial heat produced in a muscle by stimulation is not dependent merely upon the number of fibres stimulated, but also upon the amount of shortening and the tension under which the shortening takes place. In mathematical terms, and rather inaccurately,

$$H = in + kTs.$$

This means that H, the total energy liberated as a result of the contraction, is equal to the sum of two products, the factors of the first

being i, the heat produced by the isometric contraction of a single fibre and n the number of fibres stimulated and the factors of the second product being T, the tension of the muscle during the shortening, s the amount of shortening, and a constant, k, with a value usually between 1 and 2. In a later paper reasons will be given for believing that when the complications due to the lowering of the weight in relaxation can be properly allowed for the value of k is very nearly 1, *i.e.* the excess heat produced is very nearly equal to the work done.

The general nature of the muscular mechanism as elucidated by these experiments can be made clear by an analogy to an electric motor in which the energy used increases with the work done in an entirely automatic manner depending upon the intrinsic properties of the motor. When the load on the motor is increased it slows down, the back E.M.F. which it develops is decreased and more current passes through the coils. Conversely when the motor runs idle it develops a large back E.M.F. so that the current becomes very small. The necessity of providing a muscle with a mechanism of this general type makes new demands upon theories of muscular contraction which at present neither the surface tension nor the water absorption theories seem able to meet.

Summary.

1. The older work on the heat production of muscles allowed to shorten has been repeated and extended with the use of the sartorius muscle of the frog instead of the gastrocnemius. Quite different results have been obtained which are apparently due to differences in the anatomical structure of the two muscles.

2. On account of the greater anatomical simplicity of the sartorius muscle it is regarded as a better example of a single muscle fibre and hence greater theoretical significance is claimed for the results obtained with it. Consequently important modifications of some of the fundamental and accepted principles of muscle physiology have become necessary.

3. When a muscle lifts increasing weights to a constant height, the increase in heat obtained is roughly proportional to the tension under which the muscle shortens (*i.e.* the weight).

4. When a muscle lifts the same weight through increasing heights the increase in heat obtained is roughly proportional to the amount of shortening.

5. Since the product of tension during shortening and the amount of shortening, is work, the increase in heat becomes roughly proportional to the work.

THE REVERSION OF HÆMOLYSIS. By R. BRINKMAN AND A. v. SZENT-GYÖRGYI.

(From the Physiological Institute, Groningen.)

By the term reversion of hæmolysis we understand the reappearance of apparently normal red corpuscles in hæmolysed blood, so that normal blood which had been completely hæmolysed, is reversed for the greater part into normal blood once more. This process can easily be observed if one takes care to regulate the hæmolytic influence in such a way, that the occurring lysis is only a hæmoglobinolysis and not a stromatolysis. In most of the hæmolytic processes, including the biological ones, the effect of not too large concentrations of hæmolytics is the production of spherical corpuscles, followed by a rather sudden loss of the pigment. The hæmoglobin does not slowly diffuse out of the cell, but comes out rapidly. If the stroma is preserved in a more or less undamaged condition, it is possible to bring the hæmoglobin back to the stromata, so that all stromata are suddenly changed once more to apparently quite normal corpuscles. The "re-adsorption" of hæmoglobin takes place with the same velocity as the chromolysis.

The phenomenon may be observed in the following way. 10 c.c. of defibrinated blood are thoroughly shaken with ·02 c.c. of a pure higher fatty acid; we always used linolenic acid, because of the solubility of its Ca-soaps. The blood is then placed in the water bath at 37° and the electrical conductivity measured at short intervals. When the said concentration of linolenic acid is given, a steady increase of the resistance is always noted; when after 1–3 hours hæmolysis has become complete, the resistance has increased considerably and remains as high for 1–2 days. But if some more linolenic acid is added, the resistance suddenly decreases to nearly the serum value, and in this blood no stromata are present and reversion is not possible.

The following table gives the conductivity of the different sorts of hæmolysed blood, at 37°.

	$\lambda . 10^4$
Normal pig's blood	63
Normal pig's serum	137
The same blood hæmolysed with ·02 c.c. of linolenic acid in 2 hrs.	39
The same blood hæmolysed with ·04 c.c. of linolenic acid	125
The same blood laked by mechanical hæmolysis[1]	138

[1] By the method of D. J. de Waard. This Journ. 57. p. 195. 1923.

It is easily demonstrable, that in blood, laked by an appropriate concentration of linolenic acid, with a high electrical resistance, the stromata are all present, not very much swollen and not agglutinated. This may be seen by looking at the blood when it is still streaming under the coverslip, or in a drop of hæmolysed blood, mixed with a small amount of China ink. The presence of stromata may, of course, also be proved by fixation with methyl alcohol and staining with methyl violet.

Fig. 1 is a microphotograph of hæmolysed pig's blood which was

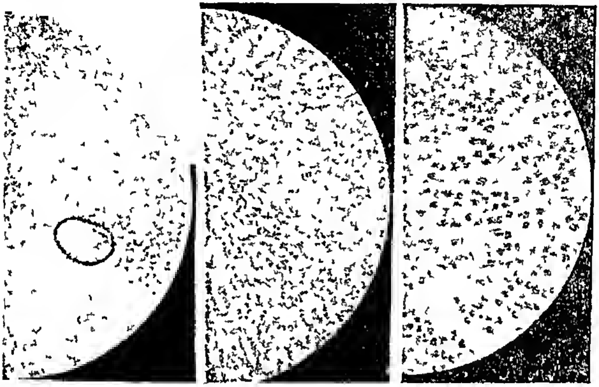

Fig 1 Fig 2 Fig. 3

somewhat diluted with serum; the contrast with the air bubble shows the hæmoglobin colour; the stromata are invisible. Fig. 2 is a microphotograph of the same blood, mixed with a trace of China ink; numerous stromata are to be seen as white spaces.

If now a small amount of isotonic saline is added to this completely hæmolysed blood, the fluid becomes clouded and bright red like normal blood. Microscopically the corpuscles have reappeared with their greenish colour; no hæmoglobin is to be seen between the cells although in the laked blood the yellow colour is observed quite distinctly between the ghosts. The reversion can directly be seen microscopically if a small drop of the laked blood on a slide is brought under the coverslip and a small amount of isotonic salt solution is run in from this side. Where the saline streams into the laked blood, the corpuscles suddenly reappear. Fig. 3

as the reversion of hæmolysis, especially that caused by hypotonia, had not formerly been noticed. Spiro is said by Rohonyi (3) to have described in 1897 the passing back of hæmoglobin and stromata on adding salt solution after hypotonia hæmolysis. Rohonyi more or less confirmed Spiro's results, but stated that the "reversal" occurred only in cells which were incompletely hæmolysed, though they were swollen to invisibility. On adding salt these cells, he said, shrank and they and the hæmoglobin in them again became visible. The phenomenon was independently observed by Adair, Barcroft and Bock (4) in blood, laked by dialysis, but apparently they considered it to be due to the assumption and subsequent loss of water by the cells, and not to dissolved extracellular hæmoglobin rejoining the stroma.

It may be mentioned that an entirely different form of "reversal" is described by Rohonyi. When he completely hæmolysed the blood by water or saponin, he found that on adding a certain amount of a substance precipitating protein, the precipitate consisted entirely of cells, containing a brown hæmoglobin derivate. We do not think that this experiment may be called a true reversion of the hæmolytic process.

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THE FREQUENCY OF DISCHARGE FROM THE SPINAL CORD IN THE FROG.

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DURING a voluntary or reflex movement the contracting muscle gives rise to a rapidly oscillating action current. The larger oscillations sometimes show a fairly definite rhythm of about 50 per sec., but if every oscillation is counted the frequency is usually nearer 120–150 per sec. and there is no true regularity in the series. Since the electromyogram was first studied by Piper and Buchanan the interpretation of these waves has been a source of controversy. According to one view each wave which appears in the muscle is due to a corresponding volley of impulses in the motor nerve fibres, so that the frequency of the electromyogram is an exact reproduction of the frequency of discharge from the motor cells of the cord. According to the other, the frequency in the muscle is usually determined by the muscle alone and represents a vain attempt to follow a much more rapid succession of impulses, or even some continuous state of excitation, arising in the spinal centres.

Without attempting a complete survey of previous work (which has been given by Forbes(1), Trendelenburg(2) and others) it may be said that the former view is strongly supported by the observations of the action currents in the phrenic nerve during respiration, first made by Dittler(3) and confirmed and extended by Gasser and Newcomer(4). Here the electrical oscillations in the nerve synchronise exactly with those in the muscle which it supplies, the frequency being about 90 per sec. It is also supported by the recent work of Bass and Trendelenburg which shows synchronous irregularities in the response taken from different regions in a large muscle. On the other hand the view that the frequency of nervous discharge is greater than the frequency of oscillations in the electromyogram gains considerable weight from the fact, shown by Buchanan(5), Forbes and Rappleye(6), and Fahrenkamp(7), that the freqnency of the electromyogram can be altered by changing the temperature of the muscle. Buchanan worked on the reflex contractions of strychninised frogs where the electric responses occur in a succession of short groups. She found that the

frequency of the action currents in each group depended only on the temperature of the muscle and not on that of the spinal cord and concluded that the electromyogram could not represent the frequency of discharge from the spinal cord. Forbes and Rappleye found that in human muscles the frequency of the voluntary electromyogram could be changed by heating or cooling the limb, and showed that this result might be explained on the supposition that the spinal centres discharge at a rate of 300–1000 per sec., a rate too rapid to be followed by the muscle. The same conclusion has been reached by Athanasiu(8) on evidence which will be discussed later.

Our interest in these conflicting results arose from an attempt made by Adrian and Olmsted(9) to determine the refractory period of the arc for the flexion reflex in the cat. Quantitative data of the rates of recovery of reflex paths seemed essential to the analysis of central conduction and we tried to obtain these by stimulating an afferent nerve with double or repeated shocks and observing the electric responses in the reflexly contracting muscle. It was found that the muscle would not respond more rapidly than 160–200 times per sec. when the afferent nerve was stimulated although it could be made to respond up to 400 times per sec. by stimulating the motor nerve. We concluded that the limiting frequency of 160 per sec. was imposed by the central part of the arc, since the muscle and the peripheral nerves would all respond more rapidly. This conclusion is quite incompatible with the view that the spinal centres normally discharge at a rate too rapid for the muscle to follow. It is conceivable that the path for the flexion reflex differs radically from that of the other reflexes, or that the use of rhythmic electric stimuli imposes an abnormally slow rate of discharge, but neither explanation seemed very likely. As a first step, therefore, we have re-examined some of the evidence which favours the higher rate of discharge from the cord.

The central fact is that in a reflex or a voluntary movement a change of temperature in the contracting muscle is found to alter the frequency of the electromyogram. We have repeated Forbes' observations on the electromyogram of human muscles in voluntary contraction before and after cooling the forearm with ice and we have found the change in frequency which he describes, but it did not seem to us that this result was conclusive evidence in favour of a very rapid rate of discharge from the central nervous system. Weizsäcker(10) and Dusser de Barenne(11) have shown recently that the frequency of the electromyogram depends on the integrity of the proprioceptor nerves from

the muscle. If these are interfered with, as by disease or by injecting novocaine into the muscle, the frequency of the oscillations is reduced. This result agrees with Hoffmann's view that the voluntary contraction is made up partly of a series of tendon reflexes reinforced from higher centres, and it suggests that cooling the muscle may affect the frequency of the electromyogram because the sensory side of the arc is impaired and not because the muscle cannot respond as rapidly as it did before. An increase in frequency on warming is not so readily explained, but here too the change in the state of the proprioceptors cannot be excluded. This objection does not apply to Buchanan's experiments on frogs, for in some of these the proprioceptor arc was destroyed by cutting the dorsal roots. These experiments we deal with below.

Method. We used spinal frogs, the brain being destroyed from 1 to 24 hours before the records were made. When the spinal cord was to be maintained at a constant temperature the body of the frog was surrounded by coils of lead tubing through which water could be circulated. When the temperature of the cord was to be changed it was cooled by placing ice in a metal container over the lower vertebræ or warmed by concentrating the rays of a "pointolite" lamp (500 C.P.) over the same area. The amount of heating could be readily controlled by an iris diaphragm in front of the condenser lens. An approximate idea of the temperature of the cord was given by a small thermometer thrust down the œsophagus into the stomach. As the heating or cooling was greatest on the surface of the back the change of temperature of the spinal cord would be greater than that in the stomach, but a comparison of the actual temperature in the vertebral canal measured with a thermo-junction and the temperature given by a thermometer in the stomach showed a difference of not more than two degrees when a steady state had been reached.

The temperature of the muscle was controlled by fixing the legs of the frog in two double walled metal troughs through which water could be circulated. The troughs were open at the top to admit the non-polarisable electrodes connecting the muscle to the galvanometer. The surface of the trough was insulated with shellac and the limb was kept just out of contact with it. With this arrangement the temperature of the muscle became very nearly constant when water at a given temperature had been circulating for 20 minutes; it was measured by a thermometer with a small bulb pressed against the surface of the muscle: in control observations this was found to give a reading identical with

that obtained from a thermo-junction thrust into the substance of the muscle.

The contractions whose action currents were to be recorded were produced either by pinching the fore-limbs or the opposite hind limb or by electrical stimuli to an afferent nerve, usually the opposite sciatic or one of the dorsal roots of the same side. The stimuli were break induction shocks from a coreless coil delivered by a rotating contact breaker previously described (12). The muscles used were the gastrocnemius or the hamstrings or adductors of the thigh. The gastrocnemius was isolated except at its origin and the electrodes (Lapicque type) were applied to the middle of the muscle and to the tendon. When the thigh muscles were used they were not isolated and the electrodes were merely fixed to the surface of the muscle. With such an arrangement the action currents might have been derived from more than one muscle, but it was found that the contraction of muscles other than that under the electrodes produced little or no effect on the record. The action currents were recorded with the string galvanometer on cinematograph film moving at a speed of about 15 cm. per sec. The usual tests were made to detect the presence of artefacts in the record due to the spread of the stimulating current.

We had two main difficulties to contend with. The first was that the state of reflex excitability did not remain constant during the lengthy periods needed to pass from one temperature to another. This applies more especially to the reflexes produced by stimulating an afferent nerve, but the variation was serious only during the breeding season. The other difficulty arose in counting the oscillations in the electromyogram. These vary greatly in size and it is often difficult to decide whether a large wave does not mask one or more smaller ones near it. In stating our results we have given (*a*) the total number of oscillations per sec., counting every rapid excursion of the string, however small, as a wave, and (*b*) the number of "large waves" per sec. For the latter we have counted every wave which is more than half the size of the largest wave in the record. The choice of this standard is quite arbitrary but the significance of these figures will appear later. The interval between successive oscillations is extremely variable and in the frog we have never seen anything approaching the almost regular 50 per sec. rhythm which is sometimes present in the human electromyogram. At the same time the total number of waves counted in successive tenths of a second does not usually vary by more than 20 p.c. Fig. 1 shows a typical electromyogram of the adductors of the thigh in a spinal frog

at 15·5° C. stimulated by pinching the fore-limbs, and this gives a good idea of the degree of irregularity usually present.

Effect of altering the temperature of muscle. In this series of experiments the temperature of the spinal cord remained constant and that of the muscles was changed. In some, a single muscle was brought successively to different temperatures, in others one leg was cooled and the other was warmed and the action current was led from them alternately. In the first experiments the muscles were examined at two temperatures, one above and the other below that of the cord and in these there was generally a decided difference in the frequencies of the electromyograms. As Buchanan had previously shown, this difference was present after the dorsal roots from the muscle had been cut. For example in a frog with the 8, 9 and 10 dorsal roots cut on both sides and the action currents led from the two gastrocnemii, one warmed to 22° and the other cooled to 10°, the body being at 15°, the reflex contractions obtained by pinching the fore-limbs gave an electromyogram of 100–120 oscillations per sec. for the warm muscle and 70–90 for the cold. The differences were evident but they were not very great and in some experiments it was by no means certain that they existed at all. A survey of the records and a comparison with Buchanan's results suggested that the difference in frequency was greatest when the muscle was cooled to a temperature much below that of the spinal cord. This had been anticipated on theoretical grounds. Beritoff(13) in his analysis of the frog's electromyogram has pointed out that the limiting frequency of response in the reflex arc will be determined by that part of the arc in which the refractory period is longest; the muscle might become the slowest component of the arc if it were cooled although it was not so when the whole arc was at one temperature. He finds that in the summer frog the maximum frequency which the muscle can follow when the motor nerve is stimulated is about 200 per sec. The average frequency in the reflex electromyogram is 100 to 120 per sec. If this represents the rate of discharge from the spinal centres a change in the temperature of the muscle will not affect the frequency of the electromyogram unless the muscle is cooled down to a temperature at which it will no longer respond to 120 impulses per sec. As soon as this temperature is reached the frequency of the electromyogram will be determined by the ability of the muscle to respond, and not by the rate of discharge from the cord.

If the change in frequency recorded in Buchanan's and our experiments is to be explained in this way we ought to find (*a*) that warming the muscle above the temperature of the spinal cord should not alter

the frequency, and (*b*) that cooling it should not do so until the temperature of the muscle is such that it would no longer be able to respond to impulses reaching it with this frequency from the motor nerve. It would be difficult to obtain direct evidence on the latter point as it would involve bringing the muscle to many different temperatures, but it can be shown that a small reduction of temperature is quite enough to make the muscle unable to follow the discharge from the cord although the frequency of this is no greater than that of the normal electromyogram. In Table I we have compared the frequency of the reflex electromyogram in the gastrocnemius with the maximum frequency of response of which the muscle was capable when the motor nerve was stimulated. The data are taken from five frogs all at a uniform temperature throughout.

TABLE I.

Exp.	Temp. ° C.	Frequency of reflex electromyogram per sec.	Maximum frequency of response in muscle per sec.
1	10	80– 90	120
2	10	80–100	120
3	14	100–120	160
4	14	120	160
5	13·5	100–130	160

Exps. made in November; reflex contraction produced by pinching; dorsal roots not cut. Motor nerve afterwards stimulated *in situ* to give column 4.

It will be seen that the muscle is normally responding at a rate which is fairly close to its maximum capacity. How much this maximum rate is reduced by cooling may be seen from another experiment in which a gastrocnemius-sciatic preparation was maintained at various temperatures in a water-jacketed chamber and stimulated with rhythmic induction shocks.

Exp. 6. (Sept.) Gastrocnemius-sciatic preparation, stimulus to sciatic.

Temp. ° C.	Maximum frequency of electric response in muscle per sec.
7	80
13	160
20	240

The maximum frequency is more than doubled for a rise of 10° C., so that a fall of 5° should reduce the maximum frequency by at least 50 p.c. A reference to Table I shows that a reduction of this extent would be quite enough to make the muscle unable to respond as rapidly as it did in the reflex contraction before it was cooled. Thus we should

expect a change in the frequency of the electromyogram on cooling the muscle through 5°, even though the frequency at normal temperatures is determined by the cord alone. At the same time the experiments bring no evidence against the alternative view that the frequency of discharge from the cord is too rapid for the muscle to follow even at normal temperatures.

Much more decisive evidence is likely to be gained by warming the muscle instead of cooling it. If the discharge from the cord is normally too rapid for the muscle to follow, warming the muscle should increase the frequency of the electromyogram and the increase should continue with rising temperature as long as there is any difference between the frequency of discharge from the cord and the frequency of response in the muscle. To test this point we have made three complete experiments (Table II) in which the body of the frog was kept at a temperature of about 10° C. and the muscle was examined at temperatures ranging between 10° and 25° C. The leads were taken from the hamstring muscles and reflex struggling movements were produced by pinching the fore-limbs.

TABLE II. Reflex contraction of hamstring muscles.

Exp.	Temperature: Cord °C.	Temperature: Muscle °C.	Reflex electric response, Frequency per sec.: Counting all waves	Reflex electric response, Frequency per sec.: Large waves only
7	9	13	130, 120, 150, 150, 140	80, 80, 50, 50, 80
	—	19	110, 100, 120, 120, 110	40, 30, 40, 50
	11	20	140, 150, 140, 130, 110	60, 40, 60, 50, 40
	9	11·5	100, 120, 110, 100, 120	50, 50, 50, 60, 60
8	10	11	130, 120, 120, 120, 110	60, 60, 40, 60, 40
	—	22	140, 130, 150, 110, 130	40, 60, 50, 40, 60
	—	12	100, 110, 110, 90, 00	40, 50, 40, 40, 50
	—	18	130, 120, 140, 140, 150	60, 40, 40, 50, 40
9	9	20	130, 100, 120, 90, 110	30, 40, 30, 30, 30
	—	12	110, 130, 00, 110, 80	30, 20, 30, 40, 30
	11	19	140, 110, 120, 130	30, 40, 50, 30, 30
	—	25	110, 140, 150, 120, 100	40, 30, 50, 40, 60
	—	11·6	120, 130, 130, 110, 130	30, 50, 40, 50, 40

Exps. made in May. Dorsal roots not cut. Oscillations counted in periods of one-tenth sec. taken at random from different parts of the record.

Table II shows that on the whole the frequency of the reflex electromyogram is slightly increased by a ten degree rise of temperature when all the waves are counted, but the difference is rarely greater than 20 p.c. and during a good deal of the records it cannot be detected at all. The average increase in frequency for a 10° rise works out at 7 p.c. There is no evidence of any change in frequency when only the large waves are counted. Typical records of these experiments are given in

the motor nerve At 7° C. the frequency is 80 per sec., at 10° it is 110 and at 20° it is 170. Figs. 2 and 3 are not strictly comparable as regards the actual rates of discharge for the reflex experiment was made in April and the other in August, but they show that a rise of temperature has a much greater effect when the responses are produced by stimulating the motor nerve at such a rate that the muscle is unable to respond to all the impulses reaching it.

These experiments make it highly probable that the frequency of discharge from the spinal cord is not as a rule too great for the muscle to follow. The slight increase which appears in parts of the record when the muscle is warmed may show that the cord sometimes discharges at a greater frequency than the muscle can follow when it is at normal temperature, but this cannot be much more than 20 p.c. above the frequency of the normal electromyogram and such a rate is evidently not constant.

The conclusion to be drawn from these experiments is that the maximum rate of discharge from the cord in the frog at room temperature is 120–150 per sec., and that the frequency of the oscillations in the electromyogram is usually identical with the frequency of discharge from the cord.

Comparison of natural with artificial stimuli. Before going further we may enquire how far the maximum rate of discharge from the cord depends on the nature of the stimulus, whether a natural stimulus such as pinching the foot may lead to a greater frequency than is given by rhythmic stimulation of an afferent nerve trunk. In a number of experiments we have used rhythmic stimuli at various rates and our results agree closely with those published by Beritoff. In winter frogs at 14° C. with stimuli up to about 50 per sec. the frequency of response in the muscle is usually greater than that of the stimulus, each stimulus corresponding to a group of 2 or 3 action current oscillations. With frequencies of stimulation between 50 and 120 per sec. the muscle response is regular and has the same frequency as the stimulus; with stimuli above 120 per sec. the response becomes irregular and the frequency remains about 120 per sec. An irregular response of this kind is quite indistinguishable from that produced by pinching. This may be seen from the records in Fig. 4 where (*a*) is the response to pinching, and (*b*) the response of the same muscle to 80 stimuli per sec. applied to an afferent nerve trunk. In this experiment the cord had been cooled to 9° so that a frequency of 80 was enough to give an irregular response.

Evidently there is no essential difference in the response to natural and artificial stimuli as long as the frequency of the latter is great enough.

In three experiments in which the response became irregular with rates of stimulation above 120 per sec., the muscle was afterwards stimulated by its motor nerve and found to be capable of a regular response with a frequency of 160 per sec. Its failure to follow reflex stimulation above 120 per sec. is therefore an added argument for the view that this limit is imposed by the conducting paths in the cord and not by the muscle itself.

Effect of altering the temperature of the spinal cord. So far the evidence is in favour of the view that the reflex electromyogram gives a fairly accurate rendering of the frequency of discharge from the spinal cord. There is however a very serious objection to this view. Buchanan showed that cooling the spinal cord in a frog did not alter the frequency of the "wavelets" of a strychnine convulsion although cooling the muscle did so. She pointed out the difficulty of reconciling this with the view stated above and so far as we are aware the objection has never been met. A reduction in the temperature of the cord would be almost certain to increase the refractory period of the central conducting paths and to lower the maximum frequency of discharge from the cord. Such a reduction has been shown by Garten(15) in the discharge of the nerve cell which activates the electric organ of Malapterurus. Here a fall of 10° C. reduces the frequency of discharge from the cell to a third of its former value. Since there is no such change in the reflex (strychnine) response of the frog's muscle when the cord is cooled, the most likely explanation is to suppose that the frequency of discharge from the cord is too rapid to be followed by the muscle even though the cord is at a low temperature. This conclusion is in flat contradiction to the results given in the preceding section. We have repeated the experiment of warming and cooling the spinal cord and our results agree on the whole with Buchanan's. The contractions were provoked by pinching; strychnine was given in a few experiments to increase the excitability of the cord but as a rule good reflex contractions were obtained without it. The muscle was kept at a temperature equal to or slightly above that of the cord when warmed; so that, according to our previous results, the muscle should be able to respond to all the impulses reaching it. The results of five of these experiments are given below in Table IV. It is true that in most cases there is a small increase in total frequency when the cord is warmed; in some it is considerable (*e.g.* Exp. 15), in some it is present in parts of the record only (Exps. 13 and 14); but the

fibres. The simplest explanation is that the small waves are due to the activity of a few only of the muscle fibres and that the greater part of the muscle only comes into play during the larger waves. When the cord is warm (Fig. 5 (*b*)) the greater part of the muscle is activated much more frequently since the large waves are much closer together. It follows that when the cord is warm the spinal centres send out volleys of impulses in rapid succession, each volley being a discharge from the greater part of the motor centre and occupying most of the fibres in the motor nerve. When the cord is cooled the main volleys are separated by much longer intervals and in between the centre keeps up a straggling, independent fire from a few units at a time. This straggling fire may be produced by some of the arcs in the spinal centre which have very much shorter time relations than the majority, or it may mean that the cooling has interfered with the unity of action of the centre so that its different parts no longer discharge synchronously. The small waves would then be due to a discharge from nerve cells which were too late to take part in the main volley, either because of a longer latency or because they were out of touch with the other units. In any case it is clear that the effect of cooling the cord is to produce a great reduction in the frequency of the large waves.

The results of cooling the cord (if our explanation is correct) can be shown diagrammatically as in Fig. 7. The tracings above the diagram are copied from a record of the electromyogram with the cord hot and cold (Exp. 18), and the diagram gives the suggested analysis of these records. The lower lines represent the discharges occurring in each motor neurone, or in each group of muscle fibres innervated by a single neurone, and the line above shows how these would add together to give the electric response of the whole muscle. When the cord is cold four neurones are represented as responding synchronously and three more at the same frequency but out of phase with the rest. When the cord is warm five neurones respond synchronously at one and a half times the frequency and two are out of phase. The resulting electromyograms have the same number of oscillations per sec. in either case, but when the cord is warm the large waves are one and a half times as frequent as they are when it is cold.

The foregoing experiments have shown that cooling the cord does cause an appreciable change in the character of the electromyogram. This change can be explained without assuming that the cord discharges too rapidly for the muscle to follow and it is extremely difficult to see how it could arise if this view were correct.

There is, however, another test which may be applied; the reflex contractions may be produced by rhythmic stimulation of an afferent

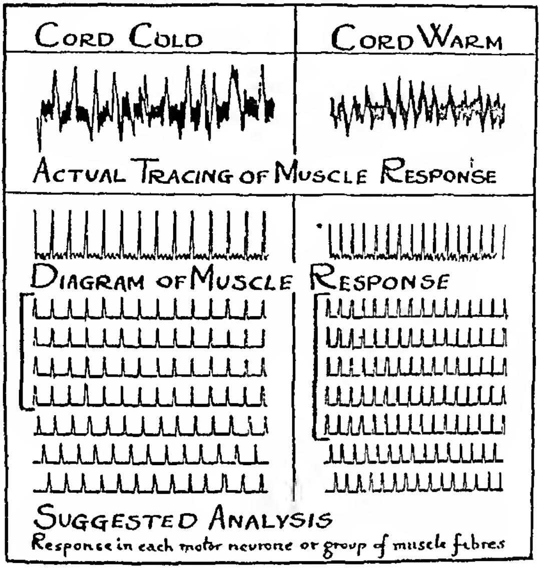

Fig. 7.

nerve instead of by pinching the skin. With the cord at a normal temperature, if an afferent nerve is stimulated with 120 shocks per sec. the reflex electromyogram will show a regular series of action currents with a frequency of 120 per sec. If the stimulation is more rapid the response becomes irregular but does not increase in frequency. We have supposed that this limiting frequency of regular response is imposed by the spinal centres and not by the muscle; if so, the limiting frequency should be very much reduced when the cord is cooled. If the cord can really respond much more rapidly than 120 per sec. and the frequency of the electromyogram is due to the muscle, then there is no reason why it should be reduced when the cord is cooled.

We have made three experiments on the same lines as those in Table IV with the difference that the reflex contractions were produced

by rhythmic stimulation of an afferent nerve (usually the opposite sciatic) at various rates from 32 to 320 per sec. A typical result is shown in Fig. 8. In Fig. 8 (*b*) the muscle is at 20° and the cord at 19°; the opposite sciatic was stimulated 80 times per sec. and the muscle gives a regular series of responses at the same frequency. In Fig. 8 (*a*) the muscle is at 19° and the cord has been cooled to 4°. The response to 80 stimuli per sec. is now an irregular medley of small waves with a few much larger, in fact it closely resembles the electromyograms in Figs. 5 and 6 where the cord was cooled. The total number of waves per sec. is actually greater in Fig. 8 (*a*) than in (*b*), but it is clear that the cord at 4° is no longer able to give a regular series of discharges at 80 per sec. At this temperature stimuli at 32 per sec. to the afferent nerve gave a regular series of responses at 32 per sec. in the muscle, but higher rates of stimulation were not followed.

Table V shows the maximum frequency of stimulation which will give a regular series of responses at different temperatures of the spinal cord. In all the experiments the response is counted as regular as long as it exhibits the rhythm of the stimulus; at low frequencies of stimulation the response to each shock may be multiple, but the record shows a definite rhythm and is quite unlike the irregular response given by more rapid stimulation. The table shows also the "Irregular frequency," *i.e.* the total number of waves per sec. in the electromyogram when the response is no longer regular. Like Table IV it confirms Buchanan's observation that the frequency of the electromyogram does not change

TABLE V. Spinal frog: rhythmic stimulation to afferent nerve at different frequencies.

	Temperature		Reflex electric response	
Exp.	Cord °C.	Muscle °C.	Maximum regular frequency per sec.	"Irregular frequency" per sec.
19	19	20	120	100–120
	8	19	48	110
	20	19	160	120–150
	4	20	32	100
	20	19	160	120–150
20	11·5	20	96	100
	7	20	doubtful	100–120
	16	20	128	120–140
	21	20	160	130–150
	7	20	48	100–120
21	13	21	80	100–120
	19	21	120	100–130

The following stimulation frequencies were used for testing: 32, (40), 48, 64, 80, 96, 120, (128), (144), 160 and various higher values. The figures in the third column are accurate only as between these values. The frequencies enclosed in brackets were often omitted from the series.

to any great extent when the cord is cooled, but it also confirms the suggestion that there is a great decrease in the frequency with which the centre can respond as a whole. If the figures in this table are compared with those in Table IV it will be seen that there is a very good agreement between the maximum frequency of regular response on rhythmic stimulation (Table V, column 3) and the frequency of the large waves in the response to pinching (Table IV, column 4) with the cord at different temperatures.

We conclude, as before, that the frequency of the electromyogram is determined by the spinal centres and not by the muscle, and we may add that the spinal centre is so constituted that it cannot discharge as a whole more often than 120 times per sec. at 15° C. or 32 times at 7° C., presumably on account of the refractory phase of some part of the conducting structures.

In one other respect this hypothesis is open to experimental verification. When the cord is cooled to 6° the frequency of the electromyogram may be as high as 120 per sec. when every wave is counted, but we have supposed that the individual motor nerve fibres and the groups of muscle fibres which they innervate are for the most part responding at a much slower frequency. If they are, then cooling the muscle down to the temperature of the cord will not reduce the frequency of the electromyogram even though a temperature is reached at which the individual muscle fibres would be unable to respond as rapidly as 120 times per sec. We have made three experiments in which the cord was cooled and the temperature of the muscle was varied. These are recorded in Table VI.

TABLE VI.

	Temperature		Reflex response to pinching Frequency per sec.	
Exp.	Cord °C.	Muscle °C.	All waves	Large waves only
22	4·5	11	110, 120, 110, 120, 120	40, 40, 20, 40, 30
	4	24	110, 140, 120, 110, 130	40, 30, 30, 30, 40
23	7	10	130, 130, 130, 150, 140	40, 40, 50, 50, 30
	6·5	15	150, 120, 140, 140, 130	30, 50, 40, 30, 40
	8·5	18	140, 140, 130, 150, 120	40, 30, 50, 40, 50
24	5·5	7·5	80, 70, 80, 60, 60	30, 30, 30, 10, 20
	* 5	7	90, 80, 100, 80, 70	30, 40, 30, 30, 20

* In this experiment the muscle was the gastrocnemius; in all the others the hamstrings were used.

Unfortunately the method of cooling the muscle by circulating cold water through the double walled trough did not allow very low temperatures to be reached. Nevertheless in Exp. 23 it will be seen that the

muscle at 10° gave a reflex response with a frequency as high as 150 per sec., and in Exp. 24 the muscle at 7° gave a frequency of 100 per sec. Both these values are well above the average for the maximum frequency of response of a muscle at that temperature when the motor nerve is stimulated and every fibre is brought into play by each stimulus, though they are not outside the extreme limits which are sometimes found. The experiments are therefore scarcely conclusive, though the high frequencies obtained in the reflex contraction do suggest very strongly that the whole muscle is not in action during each wave of the electromyogram. Incidentally the figures in this table agree with those in Table II in that the frequency does not alter when the temperature of the muscle is raised.

Remarks. The experiments just described are of interest in connection with the recent work of Athanasiu(8). From a study of the electromyogram of different muscles (mammalian as a rule) he concludes that every record is made up of oscillations of two different origins, large waves of frequency from 70 to 150 per sec. due to the activity of the muscle and small waves of frequency from 300 to 500 per sec. due to the passage of impulses in the motor nerve fibres. Our own records of the normal electromyogram (of the frog, the cat, and man) show occasional clusters of very small waves of high frequency, but we have never been able to make out any clear separation of two such types of wave as Athanasiu describes, since there are always many of intermediate size. It should be added, however, that we have not made any detailed statistical examination of our records. But it is noteworthy that the records in which two such groups of waves are most evident are those in which the spinal cord was cooled (*e.g.* Figs. 5 and 6). Here the large waves are less frequent and are separated by groups of very much smaller ones. We have suggested above that these small waves are due to the activity of only a small part of the muscle and that they are produced by a discharge of impulses from a few of the neurones in the spinal centre, which can only discharge as a whole at a much lower frequency. According to Athanasiu's explanation the small waves should be caused by impulses in the nerves and the large waves by the muscle, which is unable to respond to every impulse that reaches it. But the results given in Tables IV and V show that it is cooling the cord and not the muscle which reduces the frequency of the large waves in the electromyogram; it is very difficult to see how this result could be achieved if Athanasiu's explanation is correct. Cooling the cord should reduce the frequency of impulses in the nerve, but if this is still

too rapid for the musole to follow, tho effect would be either to leave the muscular response unchauged or else to make its frequency increase. This follows from tho fact that if a nerve is stimulated at a frequency too great to bo followed by tho muscle, the only effect of an increase in the frequency of stimulation is to cause a reduction in that of the musele response, presumably becauso there is a greater interferenec betwcen suceessive impulses at the nerve ending. This result is shown very clearly in an experiment of Athanasiu's (16), and we have observed it repeatedly. Thus, if the small waves are due to the nervous impulses and the large waves to the musclo we should expect to find that cooling the spinal cord would reduce the frequency of the former and increase or leave unchanged that of the large waves. The fact that the frequency of the large waves is greatly reduced by cooling the cord is therefore definitely opposed to Athanasiu's conception of the electromyogram if we have understood it correctly. It is indeed difficult to imagine any explanation for the reduced frequency of the large waves as long as we suppose that this is not identical with the frequency of discharge of the spinal centres.

The conception of the electromyogram to which we have been led has some important consequences. We have supposed that the centre usually responds as a whole, sending out repeated volleys of impulses from most or all of the motor neurones which are included in it, but that in certain conditions (*e.g.* when the cord is cooled) small groups of neurones may discharge independently of the main volleys. A possibility of this kind was first suggested in 1877 by Brücke to account for the very small electrical effects observed in voluntarily contracting muscles as compared with those given by an artificial tetanus. It is, however, fairly clear that under normal conditions the responses in the centre must be more or less synchronous. Unless they were we should be most unlikely to find electromyograms showing the least approach to a definite frequency. But if the small waves in the electromyogram are due to the activity of only a few neurones when the cord is cooled, the same thing may occur to some extent at a normal temperature also. Piper considered that the spinal centres in man tended always to respond at a definite frequency which varied to some extent with the muscle concerned and was in the neighbourhood of 50 per sec., and he supposed that any departure from this rhythm was due to neurones out of phase with the main body. This view has been generally abandoned, since a large number of electromyograms show no trace of a dominant rhythm of 50 per sec.; but it may still be true, as Piper

supposed, that the more irregular the record the greater the lack of coordination between the different neurones which supply the muscle. If so a study of the electromyogram in different conditions may lead to valuable information about the general make-up of a reflex "centre" in the cord.

Further discussion of the mammalian electromyogram would be premature. The experiments we have described have been confined to frogs and they do not prove that the mammalian spinal cord cannot discharge impulses at a frequency too rapid for the muscle to follow. In the frog the maximum frequency of discharge from the cord is not far off the maximum frequency of response in the muscle and it may perhaps surpass it in other animals. At the same time the view that it does so has been based partly at least on the temperature effects in frogs and we have found that these are in reality best explained on the view that the discharge from the cord has the same frequency as the electromyogram.

Summary.

The theory that in reflex or voluntary contraction the nerve centres send out impulses at a greater frequency than that shown in the electromyogram has been submitted to examination. This view has been based on the effects of local alterations of temperature which suggest that the frequency of the electromyogram is determined by the muscle rather than the cord. On the other hand a serious objection arises from the fact that a muscle stimulated reflexly will not give a regular response at a frequency greater than 160–200 per sec. (in a mammal), whereas it will respond regularly at 300–400 per sec. when the motor nerve is stimulated.

We have examined the effects of temperature alterations in spinal frogs and we find that they do not support the view that the discharge from the cord is too rapid for the muscle to follow. If a frog's muscle is warmed through 10° C., the average increase in the frequency of the reflex electromyogram is less than 10 p.c., whereas if the muscle is responding to a very rapid series of stimuli to the motor nerve (600–800 per sec.) a rise of 10° will increase the frequency of response by 70 p.c. Cooling the muscle may reduce the frequency of the reflex electromyogram, but this, in the frog, is the inevitable result of the prolongation of the refractory period of the muscle.

It was shown by Buchanan that altering the temperature of the spinal cord did not alter the frequency of the "wavelets" in a strychnine

contraction. In general agreement with this we find that cooling or warming the cord usually causes but a slight change in the frequency of the reflex electromyogram. But there is a considerable change in the form of the response, for the number of large waves per sec. varies very clearly with the temperature of the cord though the frequency may be unaltered when waves of every size are counted. The explanation we take to be that the large waves represent the simultaneous discharge of impulses from the majority of the nerve cells and consequent contraction of the majority of the muscle fibres, and that the small waves represent the discharge from a small number of nerve cells out of phase with the rest. On this view the frequency with which the centre discharges as a whole is represented by the number of large waves, and this varies with the temperature of the spinal cord and not with that of the muscle. This view agrees more or less with Piper's original interpretation of the electromyogram. It is supported by the results of rhythmic stimulation of afferent nerves, etc.

We conclude that in the frog the nerve cells in the spinal cord do not discharge impulses at a frequency greater than about 120 per sec. at 15° C.

The expenses of this investigation were in part defrayed out of a grant from the Government Grants Committee of the Royal Society to one of us. (E. D. A.)

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THE EXCRETION OF CHOLIN IN THE URINE.

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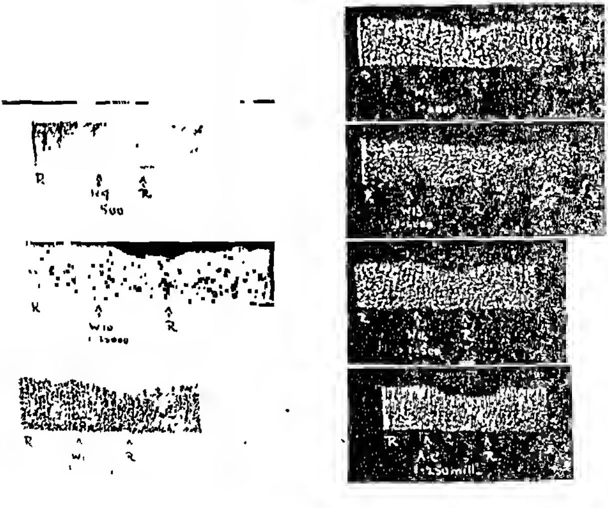
THE fate of cholin in the body has never been properly investigated. With regard to its excretion in the urine the following statements are to be found: (i) Cholin given in doses of 1 gm. to rabbits subcutaneously or by the mouth does not appear in the urine (von Hoesslin(1)). (ii) The urine of rabbits fed on lecithin contains no cholin (Franchini(2)). (Both quoted from Barger's *Simpler Natural Bases*(3).) (iii) After intravenous or intracerebral injection of 0·3 to 0·7 gm. of cholin none appears in the urine (Donath(4)). In this paper Gumprecht is quoted as having detected it after administration of doses of 1 gm. subcutaneously. (iv) In man when lecithin is taken by the mouth in doses of 5 to 10 gm. per day there is a quantitative excretion of cholin in the urine (Berg(5)); no details of method of estimation, etc. are given. (v) Guggenheim and Loeffler(6) state that a small proportion of cholin given intravenously appears in the urine but not if the administration is subcutaneous. (Doses up to a total of 2·2 gm. in 18 days.)

It appears, therefore, that there is little agreement on the subject and this may be partly explained, especially as regards the negative results, by the great difficulty of detecting cholin in minute quantities by chemical means. Indeed it has been stated that there is no satisfactory chemical test for cholin. Guggenheim and Loeffler(6) employed the *biological* test after conversion of the cholin into acetylcholin as first described by Reid Hunt(7). In their paper they outline a method for the formation of the acetyl derivative which I have found to give uniformly satisfactory results. The biological test on the frog's heart as described by Reid Hunt(8) and by Fühner(9) is perfectly trustworthy if its limitations are appreciated.

In investigating the excretion of cholin in the urine I used the hydrochloride for administration to rabbits and rats, (i) subcutaneously, (ii) intravenously, (iii) by the mouth. In no case were the doses employed sufficiently large to produce severe general effects and slight symptoms were observed in only one case. The method adopted was, in general, as follows: the animals were kept in cages on a liberal diet, freely supplied with green stuff or water and the urine was collected periodically. The

total urine for the given period (24 hours in the case of rabbits) was evaporated to dryness on the steam bath, the dry residue ground up with sand and placed in a stoppered bottle with absolute alcohol for a minimum of 24 hours. The alcoholic extract was filtered and evaporated to dryness on the steam bath. The last part of the process was conducted in a stout walled test-tube and the product finally dried in a desiccator. A crystalline mass was thus obtained to which was added a small quantity of acetyl-chloride. The tube was sealed and immersed in a water bath at 100° C. for two hours, then opened and heated on the steam bath to drive off the acetyl-chloride. The contents were extracted with a few c.c. of water, neutralised with NaOH and made up to 50 c.c. with frog Ringer. Suitable dilutions of these 50 c.c. products were tested by perfusion of the excised heart of the frog.

It is of course obvious that the 50 c.c. of solution obtained by the above process contains many substances extracted by the alcohol from



a *b*

Fig. 1. Action on the perfused heart of acetylated products of urines, showing approximately the same activity in the dilutions given in Exp. 1. Bottom trace, acetyl-cholin 1–250 millions. R=Ringer. The arrows mark the points at which perfusion of the fluid to be tested was begun or the return to Ringer after the test.

the urine, that it is in all probability not isotonic with pure Ringer, and that it is possible that it will have some effect on the heart apart altogether from any acetyl-cholin which it may contain. Such objections are answered by the fact that the original solutions are diluted until an effect is just observable and that a comparison is instituted between urinary extracts made under identical conditions except that some are before and some after the administration of cholin. The precaution was also taken of adding small amounts of cholin to certain specimens of normal urine before extraction (see protocols of experiments). All solutions were adjusted to a uniform pH and those belonging to one experiment were of course tested in a batch on one heart. No comparison can be instituted between results obtained on different hearts as these vary considerably in their response. The following are some illustrative experiments:

The dilution given is the maximal dilution of the extract (50 c.c. see text) which gave an eff

Rabbits

Exp. 1. Weight, 2·6 kgm.

Time in days	Total urine c.c.	Dilution
0	76	500
1	140 + 2 mgm. cholin	25000
200 mgm. cholin subcutaneously		
2	200	50000
3	82	4000
4	203	4000
5	41	500

Exp. 3. Weight, 1·8 kgm.

0	360	1000
1	295	1000
2	120	500
40 mgm. cholin into auricular vein		
3	210	20000
4	200	1000
5	145	500
6	130	500
	130 + 5 mgm. cholin	20000

Exp. 2. Weight, 2·1 kgm.

Time in days	Total urine c.c.	Dilut
0	140	10
1	495	10
2	480	10
50 mgm. cholin subcutaneously		
3	315	30
4	360	20
5	335	20
6	220	20
7	330	20
8	155 + 2 mgm. cholin	100
9	420 + 4 mgm. cholin	200

Exp. 4. Weight, 2·0 kgm.

0	250	1
1	295	1
	295 + 10 mgm. cholin	20
50 mgm. cholin into auricular vein		
2	435	5
3	115	1
4	275	1

* Half of the urine only was acetylated. In t other half the dilution required was 10.

Rats

Exp. 5. Urine collected before and after giving cholin.

3 days before	40	100
25 mgm. cholin subcutaneously		
3 days after	32	2500

Exp. 6. Urine collected for 4 days.

Control	27	10
"	29	5
108 mgm. cholin in drinking water	36	5
111 "	20	10

Negative results were also obtained when 1 and 200 mgm. respectively were given in t food.

NOTE. In the case of the rat experiments I wish to express my obligation to Miss P. S. Hende son for the loan of the animals and for attending to the collection of urine and the feedir All the animals used were about the same size (100 gm.).

These results indicate clearly that after injection of cholin a substance is present in the urine which after acetylisation has a marked effect on the frog's heart and there is no room for doubt that the substance is actually cholin. The activity of post-injection specimens is lost on standing (as is the case with pure acetyl-cholin) and approximates to that of the pre-injection specimens which show little difference. Hence the activity of the latter is not due to acetyl-cholin. It is probably caused by sodium acetate formed at the neutralisation of the (decomposed) acetyl-chloride during preparation. At least the addition of sodium acetate in relatively small amounts to Ringer produces a similar effect.

Having regard to the uncertainty attending a biological test the uniformity of the qualitative results was remarkable. In no experiment (and several are not detailed) was the effect absent. In most of the experiments I determined the total solids of the urine and the weight of the alcoholic extract, but as no relation was apparent between these factors and the degree of activity the figures have been omitted as valueless. The results are, I think, very roughly quantitative but the method employed is too imperfect to trust it in that respect. In order to avoid the extraction of so much adventitious matter I tried one or two solvents other than alcohol (ether, chloroform, etc.) but these failed to take up cholin added to specimens of urine.

Conclusions.

1. After subcutaneous injection of cholin in doses of about 25 to 250 mgm. per kilo. a small percentage appears in the urine.

2. After intravenous injection of cholin in doses of about 20 mgm. per kilo. a small percentage appears in the urine, but more than in 1.

3. Elimination appears as a rule to be nearly complete within 24 hours.

4. If cholin is present in normal urine it is in exceedingly minute amounts.

5. After oral administration of cholin in doses of 1 to 2 gm. per kilo. (rats) none appears in the urine.

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Sodium sulphate.

	Rates of urine after/before	Glucose concs. in urine after/before	Total excretion of glucose after/before	Glucose concs. of serum after/before	Actual rates of urine before injection in c.c. per min.	Actual p.c. concs. of glucose in urine before injection
	1·62	0·25	0·41	—	0·096	6·1
	4·7	0·079	0·38	1·1	0·027	10·9
	9·7	0·12	1·1	0·92	0·021	14·1
	3·0	0·41	1·23	0·91	0·215	4·35
	3·8	0·0	0·0	0·88	0·013	6·5
	1·43	0·55	0·79	1·02	0·15	6·0
Second injections	2·87	0·71	2·06	—	0·068	2·58
	2·2	0·87	1·96	—	0·021	1·25

Normal saline.

	Rates of urine after/before	Glucose concs. in urine after/before	Total excretion of glucose after/before	Glucose concs. of serum after/before	Actual rates of urine before injection in c.c. per min.	Actual p.c. concs. of glucose in urine before injection
	0·85	1·09	0·92	0·87	0·067	10·7
	2·5	0·97	2·47	0·92	0·091	6·9
	1·27	0·83	1·14	—	0·059	13·1
	0·51	1·14	0·61	1·08	0·081	13·4
Second injections	0·86	0·87	0·74	0·88	0·021	12·9
	3·5	0·71	2·5	0·87	0·014	6·5
	1·5	0·81	1·17	1·01	0·051	15·5
	2·94	0·92	2·75	—	0·049	14·1
	2·4	0·86	2·06	—	0·208	1·36

Urea (in normal saline)

	Rates of urine after/before	Glucose concs. in urine after/before	Total excretion of glucose after/before	Glucose concs. of serum after/before	Actual rates of urine before injection in c.c. per min.	Actual p.c. concs. of glucose in urine before injection
	1·6	0·43	0·69	—	0·083	8·4
	1·2	0·63	0·75	—	0·209	7·1
Second injections	1·27	0·29	0·36	—	0·06	9·2
	1·15	0·64	0·75	—	0·209	5·42

The contrast between the effect of the normal saline and isosmotic sulphate is marked. Fig. 1 illustrates the third sulphate experiment.

There is in all the sulphate experiments (except the two second injections) a large fall in the concentration of the glucose in the urine, and generally a pronounced fall in the total output of glucose. On the other hand in the normal saline experiments—with comparable rates of flow—there is only a slight change in the concentration of glucose in the urine, and the total output of glucose rises in proportion to the diuresis. In the case of the two second injections with sulphate the urinary glucose had not returned to the initial value when the injection was given. Phosphate (6 exps.), urea (4 exps.), strong sodium chloride (6 exps.) behaved similar to sulphate first injections. Of 15 injections of sodium iodide all but three behaved like sulphate and among four bicarbonate experiments all but one.

If the relative alterations of urinary sugar concentration be graphed against the relative alterations in the rate of flow the results appear to fall into three groups (Fig. 2). The upper group contains the experiments of the normal saline type, while the others are divided between the other two groups. The lower two groups, as seen from Fig. 3, seem to fit in

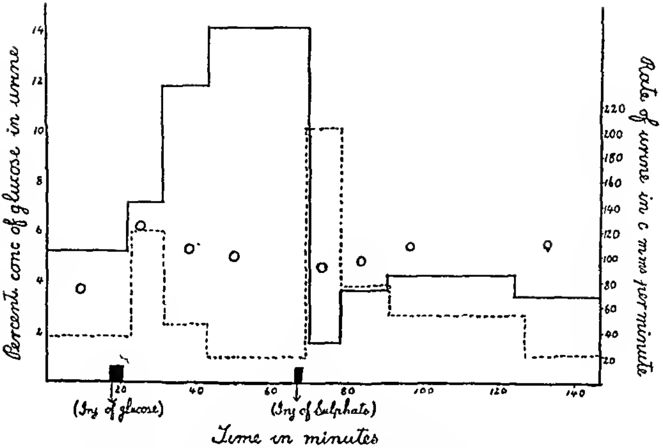

Fig. 1. Thick line sugar concentration of urine. Circles, sugar concentration of serum × 10. Dotted line, rate of flow of urine.

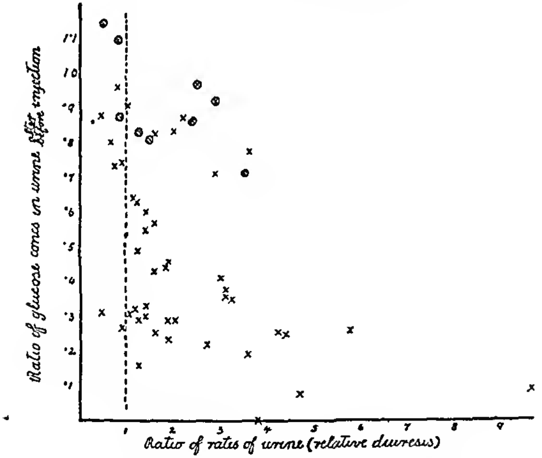

Fig. 2. The observations enclosed in circles are from normal saline injections.

with an empirical formula $C \times \sqrt{R} = K$ and $2K$, where C is the relative alteration in sugar concentration and R the relative diuresis.

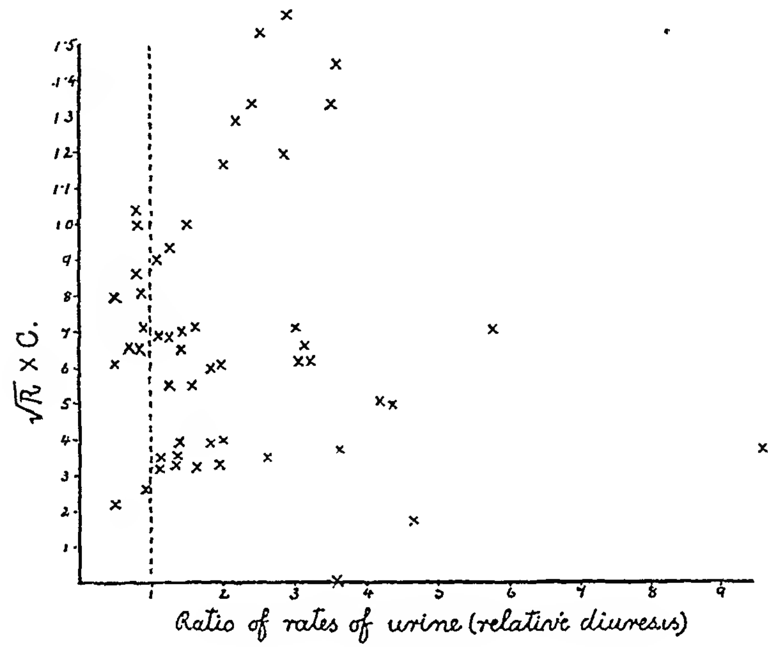

Fig. 3.

For the same relative diuresis there is a much greater fall in the glucose concentration in the urine with substances of the sulphate class than with the chloride class. In regard to the total output of glucose there is in general a fall in the output of glucose in the sulphate group when the diuresis is not marked. If the rate of urine flow remains unchanged by the injection (cf. Fig. 3) the output would in the sulphate group fall to one-third or two-thirds of previous value. The fact that the marked glucose reduction is caused by so many substances seems to rule out a purely membrane effect (9), for it is, difficult to see how urea

Physiological saline, which does not cause any pronounced alteration in the concentration of glucose in the urine, was the only fluid in the series examined at all comparable in composition to blood plasma. It seems legitimate therefore to assume that the alterations in the urinary sugar caused by other injections are due to a removal of sugar that would otherwise be excreted.

The glucose concentration as a rule returns to its initial value about half an hour after the injection; before, consequently, the injected material has been completely removed. The reduction in sugar may be due to some initial utilisation of glucose in the excretion of the foreign substance.

That the results obtained with these substances fall into two groups, one indicating twice the activity of the other, might conceivably be due to independent activity of the proximal and distal portions of the convoluted tubules.

Summary.

Experiments on the influence of diuretics on the secretion of glucose by rabbits under anæsthesia show that:

1. Physiological saline has but little influence on the sugar concentration in the urine.

2. Sodium sulphate, phosphate, bicarbonate, iodide and urea, injected in solutions isosmotic with blood and in the same quantity (30 c.c.) as the physiological saline caused a marked fall in the glucose concentration in the urine and generally a fall in total output of glucose. Occasionally an iodide or bicarbonate injection behaved like the normal saline. Strong sodium chloride solutions behaved like sulphate, etc.

3. This fall of concentration in glucose increases according to the degree of diuresis, and its relation to the change in flow of urine follows a simple empirical law.

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THE SUPRARENAL CORTEX OF THE MALE THROUGHOUT THE ŒSTROUS CYCLE.

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THE close connection between the gonads and the cortex suprarenalis or inter-renal tissues has been for long recognised. They are both developed from the mesothelium of the genital ridge. In structure the lipoid containing cells of the inter-renal tissue is indistinguishable from that of the interstitial cells of the testes and ovaries. Their probable functional connection has been indicated by many observers. Stilling(1) found enlargement of the adrenals in male rabbits during the breeding season. He also observed seasonal variations in the adrenals of frogs—during the summer, the peripheral part of the cortex contained peculiar elements—summer cells—which atrophied when the sex glands began to enlarge. Aichel(2) noted that the adrenals were very large in animals with well-developed sex organs or reproductive instincts. He also found that there was an increase in size of the adrenals of birds and some amphibia during the breeding season. Guieysse(3) found considerable enlargement of the adrenals affecting especially the zona fasciculata in pregnant guinea-pigs. Gottschau(4) working on pregnant rabbits states that the outer part of the cortex increases in thickness at the expense of the medulla and inner zone. After castration, Schenk(5) found enlargement of the cortex, especially in zona fasciculata, and Cecca(6) states that he found that both cortex and medulla enlarge after castration.

Glynn(7) gives a very full account of the relation of changes in the suprarenals to abnormal conditions of the gonads. The changes in the gonads and more especially in the interstitial cells of the testes throughout the œstrous cycle have been studied by many investigators. References to previous work will be found in my paper in this *Journal*, vol. 53, 1919. Sir Frederick Mott(8) in an investigation of the normal and morbid condition of the testes from birth to old age, states that with the dawn and development of the sexual desire there occur two histological changes—reappearance of interstitial cells in an active state, and the accumulation in and around them of granules staining with Sudan III:

and this may be regarded as an indication of the presence of a phosphorised lipoid which may serve as the raw material from which can be built up the nuclein necessary for the active formative cell processes connected with spermatogenesis.

It therefore seemed desirable to study if changes in inter-renal tissue go on concurrently with those in the testes. The mole was selected because of its well-marked cycle and because the previous work of Tandler and Gross(9) had dealt so fully with the changes in the testes and in their interstitial cells.

Methods. The animals were trapped each month during 1920 with the exception of December when no supply was forthcoming. The posterior part of the body including the suprarenals was at once preserved in 10 p.c. formalin. Sections of uniform thickness were cut by the freezing method and stained with Sudan III or osmic acid and all the sections were kept for the same length of time in the various fluids. To prove that the parts of the sections staining with Sudan III or osmic acid were really of a fatty nature, sections were treated with ether previous to application of the stain, sections so treated were not affected either by Sudan III or osmic acid.

Results. Measurements showed that the total size of the suprarenals did not vary markedly throughout the different months but that the width of the cortex reached a maximum in March and April. For this investigation four or five animals were used each month.

CORTEX OF SUPRARENAL.

	Length mm.	Breadth mm.		Length mm.	Breadth mm.
Jan.	2 75	0 30	July	3 0	0 33
Feb.	3 0	0 38	Aug.	3 0	0 35
Mar.	3 5	0 41	Sept.	3 0	0 36
April	3 25	0 41	Oct.	2·75	0 36
May	3·2	0 38	Nov.	3 0	0 33
June	3 0	0 30			

On examining the series of sections it was found that those of the month of April showed the largest amount of lipoids. The sections of this month then were taken as a standard and the sections of the suprarenals from animals killed during each of the other months compared with them. The accompanying chart indicates the changes in the lipoid content of the suprarenals throughout the year. The values are purely arbitrary. It is seen that during the month of April the lipoid content of the suprarenal is at its maximum. This is followed by a gradual decline until October, when there is observed a slight rise, again followed

by a diminution until January. Thereafter the lipoid content is slightly increased until March when there is a marked decrease succeeded by

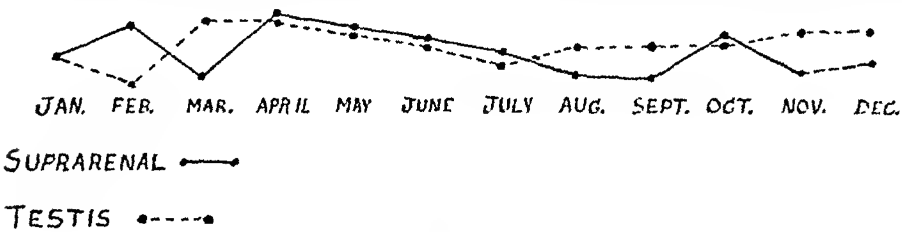

Fig. 1.

the increase to the April maximum. To advance an explanation for the decrease in the lipoids during March and the increase in October requires some reference to the changes that take place in the testes during the œstrous cycle. Serial sections of the testes were prepared from each animal and stained with iron hæmatoxylin. On examination of these it was found that the tubules were well formed with free spermatozoa in the month of March: this agrees with Tandler and Gross' observations. In April the tubules began to show signs of degeneration which continued till October. During that month the tubules showed signs of a slight regeneration and this coincided with the increase in the amount of lipoids in the suprarenal. It seems probable that the marked diminution of the lipoids in the month of March is due to a great demand made by the rapidly developing and active testes during the height of the rutting season. Millais (10) states that moles may have a second litter late in the year, which seems to indicate an original di-œstrous habit, suggested in our series by the slight regeneration of the tubules.

It would seem as if a preparatory storage of lipoids in the suprarenals took place which was not called upon by the more or less suppressed activity of the testes in the second œstrous.

These observations seem to show that the inter-renal cells play an ancillary part to the interstitial cells of the testes in the storage of lipoids which seem to be required in the production of spermatozoa. Sir Frederick Mott in the paper previously mentioned states that one of the functions of the adrenal cortex is that of storing lipoids which pass into the blood and so keep constant the supply to the reproductive organs.

I am deeply indebted to Prof. D. Noël Paton for advice and criticism throughout the course of the work.

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THE CONCENTRATION OF LACTIC ACID IN THE BLOOD IN EXPERIMENTAL ALKALÆMIA AND ACIDÆMIA.

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Macleod and his co-workers(1) have shown that administration of alkali causes an increase of lactic acid in the blood and urine, an increase of glycolysis in the blood and a decrease in the blood sugar concentration. Macleod(2) also reports some experiments on lactic acid in anoxæmia but his results are complicated by the fact that lack of O_2 was accompanied by lack of CO_2. In one set of experiments the anæsthetised animal breathed into a closed system containing lime bottles for the absorption of CO_2. It is clear from the data that there was here lack of CO_2. Moreover in one only of the experiments did the concentration of the lactic acid return to normal after the cessation of the anoxæmia. Two experiments were made on the effect of asphyxia; in one of these there was a marked rise in lactic acid so far supporting the view that the accumulation of lactic acid was due to lack of oxygen, but in the other experiment a rise in lactic acid did not occur. Macleod does not support his conclusions by analysis of the blood gases nor by the determination of the H ion concentration of the blood. Macleod considers that the accumulation of lactic acid in anoxæmia may assist in the neutralisation of the relatively increased base which results from the blowing off of CO_2 from the blood due to stimulation of the respiratory centre by the oxygen deficiency. Macleod was, however, unable to obtain any evidence that lactic acid can be produced for this purpose when there is no oxygen deficiency.

In an animal with intact nervous system it is difficult to separate the various factors affecting lactic acid concentration. Administration of CO_2 causes high blood-pressure and muscular movements; and excessive respiration designed to remove CO_2 causes a marked fall of blood-pressure and consequently a deficient circulation.

It seemed therefore desirable to test Macleod's conclusions under such experimental conditions which would allow of a better control of all the factors involved.

Method. The majority of the experiments were performed on the heart-lung preparation devised by Starling(3), defibrinated blood being used. The blood circulating in such a preparation is subject to two main changes: (1) a progressive concentration of the blood due to evaporation of water from the lungs, (2) a heavy loss of CO_2 and, in consequence, a marked movement of the *pH* of the blood to the alkaline side. Both changes are due to the excessive pulmonary ventilation unavoidable in the heart-lung preparation. The concentration of the blood was prevented by passing the air on its way to the trachea through a column of water warmed to about 50° to 60° C. The air thus saturated with water reached the lungs at a temperature of about 34° C. This simple arrangement proved quite effective. Any experiment in which the hæmoglobin concentration of the blood increased more than 10 p.c. was rejected. The blood samples were collected either from a T-piece inserted into the arterial side of the apparatus or from the venous reservoir. The blood was drawn under a thick layer of paraffin without contact with air, and a little sodium fluoride was added to prevent glycolysis(4).

All determinations were performed in duplicate and were completed either during the progress of the experiment or were carried to such a stage that no spontaneous changes could occur to vitiate the results. The following determinations were made: 1. The *p*H of the blood by Dale and Evans' dialysis method(5). 2. The CO_2 content of the blood by van Slyke's method(6). 3. The hæmoglobin concentration. A 0·5 p.c. solution of the blood treated with CO was compared in a Duboscq colorimeter with a similar solution of the first blood sample which was taken as standard (100 p.c.). 4. The blood sugar by Maclean's method(7). 5. The lactic acid of the blood by Clausen's method(8).

In certain experiments the following determinations were also made: 6. The oxygen saturation of the blood by Barcroft's method. 7. The CO_2-dissociation curve of the blood. 8. The CO_2-reaction curve of the blood. 9. The chlorides of the blood and serum by Smith's method(9). 10. The total solids of the serum.

The method of estimating lactic acid was carefully tested before the experiments were undertaken. The reaction best adapted for the estimation of small amounts of lactic acid is that exploited by von Furth and Charnass(10), whereby oxidation to acetaldehyde and CO_2 is effected by very dilute permanganate, the acetaldehyde being removed as rapidly as it is formed, trapped in bisulphite and estimated by some iodometric method. The difficulty in its application to biological material is the presence of other substances which by the permanganate treatment

yield appreciable amounts of bisulphite-binding substances. This difficulty is only overcome by some form of extraction of the lactic acid from the interfering compounds. Such treatment is, however, time-consuming and difficult where small amounts have to be determined; it would seriously limit the number of observations which could be made in any one experiment In discussing the estimation of lactic acid in blood Clausen points out that the substance in a protein-free filtrate which is the chief contributor to errors in the direct determination of the lactic acid is glucose and suggests its removal by the method of van Slyke and Fisk(11). By this method the proteins are first removed from the blood by tungstic acid and the sugar is then removed from the filtrate by treatment with copper sulphate and lime in the prescribed amounts. The v. Furth and Charnass determination is applied directly to the filtrate so obtained. It is uncertain from Clausen's paper(8) whether he adopted this method or whether he relied on that involving ether extraction. In any case he only quotes two figures to show that the two methods give concordant results.

The first point we tested was the efficiency of the sugar-removing treatment applied to mixtures of pure zinc lactate and sugar and to blood to which varying quantities of these two substances had been added. It was found that sugar was completely removed in concentration in the blood as high as 5 p.c. and that added lactic acid was quantitatively recovered. The other important question was to determine the results given by this method as compared with that involving ether extraction on various samples of fresh blood. Table I gives a few representative results from which it may be concluded that the method does yield values which are comparable with those given by the longer method and represents fairly closely the lactic acid content.

A further point is that of the recovery of lactic acid as acetaldehyde by the permanganate treatment. Von Furth and Charnass, as also Clausen, obtained a 90–92 p.c. recovery, whilst in the hands of some workers only 85 p.c. has been recovered. We have found, working with

Exp. 1.

Sample	Lactic acid added mgms.	Found: Rapid method	Found: Extraction method
1	0	35	29
1	50	82	80
2	0	47	50
2	65	109	116
3	0	52	45
3	90	138	134

amounts of pure zinc lactate comparable to the amounts of lactic acid likely to be encountered in the blood that the recovery was consistently 89–92 p.c., and we have therefore followed Clausen in adopting the empirical factor: 1 c.c $N/100$ iodine = 0·5 mgms. lactic acid (theory – 0·45 mgms.).

It was realised that the methods of von Furth and Charnass would be open to error if the amounts of acetone and aceto-acetic acid in the blood were appreciable, as the acetone would be bound by the bisulphite and determined as lactic acid. For this reason in several of the critical experiments the acetone bodies were determined independently of the lactic acid by van Slyke's method (11) and found to be not only very small but to be independent of the lactic acid changes on which emphasis is laid. The possibility that production of acetone bodies accounted for the results obtained may be confidently dismissed.

Control experiments. In the heart-lung preparation it is practically impossible to avoid over-ventilation of the lungs and, in consequence, loss of CO_2 and a shift of the pH to the alkaline side. Whilst the preparation constitutes only about 1/20 of the tissues of the animal, it is impossible to reduce the ventilation to the same degree because the oxygenation of the blood is thereby seriously impaired and, moreover, there generally results a quick development of hypostatic patches in the lungs followed by progressive acute œdema. A heart-lung preparation when performed in the usual manner may be said to be in a condition of extreme alkalæmia. It is not infrequently found that the CO_2 content of the blood may be as low as 7–10 volumes p.c., the CO_2 capacity of the serum at 42 mm. may be only 15–20 volumes p.c. and the pH may be 7·8 or still higher. All the control experiments were, therefore, under conditions of severe over-ventilation.

The first blood sample was never collected until 15–20 minutes after the circulation through the preparation had been started, so that a thorough mixture of the blood and its equilibration with the tissues might be assured. Further samples were drawn at intervals of ½–1 hour or more.

Exp. 2 is typical of the results obtained. The times given are measured from the moment when the circulation in the preparation was begun. The CO_2 content was low to begin with and continued to fall throughout the experiment, the small rise shown in the last determination can probably be accounted for by the slight concentration of the blood. The pH moved from a very alkaline value steadily towards more normal values—a movement incapable of explanation by the CO_2 content of

Exp. 2. Control exp. Arterial blood-pressure: 90 mm. Hg. Output of left ventricle: 320 c.c. per min. Temp.: 36·5° C. Heart rate: 108 beats per min. The time is counted in this and all other exps. from the moment of switching over.

Time	*p*H of blood	CO_2 content Volume p.c.	Lactic acid mgms. p.c.	Sugar mgms. p.c.	Hæmoglobin concentration	Oxygen saturation p.c.	Solids in serum p.c.
0.15	7·80	21	34	150	100	95	7·12
1.15	7·76	11	—	—	100	95	7·14
2.15	7·64	8	59	93	101	96	7·18
4.15	7·52	8	94	41	102	92	7·26
5.20	7·49	12	97	15	106	94	7·40

the blood. The lactic acid concentration rose consistently whilst that of the sugar fell. Undoubtedly the accumulation of lactic acid was an important factor in the modification of reaction of the blood and of its CO_2 capacity when exposed to 42 mm. pressure of CO_2. The CO_2 dissociation curves and the CO_2 reaction curves of the first and last blood samples are given in Fig. 1. Curve *a* is the CO_2 reaction curve

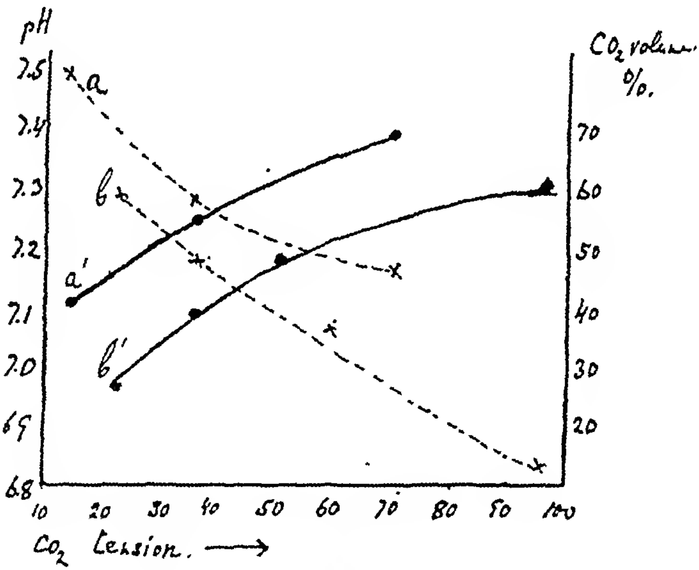

Fig. 1.

and *a'* the CO_2 dissociation curve of the first sample, while *b* and *b'* are the respective curves for the last sample. No correction has been ade for the small increase in the concentration of the blood in the l st sample. This correction would tend slightly to accentuate the di erences between the condition of the two samples of blood. It is app rent that the CO_2 carrying power and the buffering efficiency of the b ood have suffered considerably during the experiment as the rise

in lactic acid would lead one to expect. This accumulation of lactic acid in the blood during over-ventilation of the lungs has already been suggested by Dale and Evans(12). They found that animals, in which the central nervous system was intact, subjected to over-ventilation gave initial values for the *p*H of the blood sometimes as high as 9, but became less alkaline as the over-ventilation was continued. No rise in CO_2 content accompanied this change. The authors were led, therefore, by the work of Macleod and Knapp(1) to conclude that there had been, probably, an accumulation of lactic acid in their experiments. They considered that the extremely deficient circulation, which accompanied the excessive pulmonary ventilation and indicated a degree of tissue anoxæmia, was a sufficient explanation for the increase in lactic acid. The experiments reported in this communication differ from those of Macleod in that the accumulation of lactic acid cannot be attributed to anoxæmia as there was no change in the supply of blood to the tissues and the oxygen saturation varied only within very small limits. Further, there is no reason to suppose that the change in the dissociation of the oxyhæmoglobin as the *p*H moved towards more normal values with the accompanying increase in oxygen tension can explain the increase in lactic acid. The oxygen tension of the blood at the end of Exp. 2 must have been greater than at the beginning, owing to the change of *p*H, whilst the oxygen saturation remained at 95–94 p.c. This would lead one to expect that the tissues at the end of the experiment could if anything more readily have oxidised the accumulated lactic acid, but it is seen that this did not occur. This question will be discussed later in the paper.

All control experiments carried out in this manner gave substantially similar results. One point may be noted in connection with a few of them. On several occasions the blood sugar fell to indeterminable amounts before the end of the experiment, but none the less the lactic acid continued to accumulate. It would appear that the sugar of the blood was in these experiments not the only source of the lactic acid.

Effect of reducing the pH *of the blood.* The condition of alkalæmia inherent in a heart-lung preparation may be readily avoided by the addition of CO_2 to the respired air. The effect of this upon the circulation is negligible in comparison with that in an animal with intact nervous system and is mainly observable by its effect on the heart rate. If the percentage of CO_2 in the respired air be not excessive, the heart soon recovers and resumes its normal rate. If the concentration of CO_2 is high the heart beat remains considerably slower throughout the

period of administration. In order to control this factor as far as possible, small doses of adrenaline were added to the blood. 1 c.c. of 1 : 100,000 adrenaline to every 800 c.c. of blood was added every half-hour throughout the experiment. These doses hardly affected the normal heart beat but were generally sufficient to prevent the slowing due to the addition of CO_2 to the air. Control experiments performed with the addition of adrenaline in the same way showed no difference from those in which the drug was not used. Paterson(13), it may be noted, has shown that under the influence of adrenaline the heart is able to stand very high tensions of CO_2.

In our experiments the preparation was first ventilated with air for about an hour in order to allow of the accumulation of lactic acid so that the effect of the subsequent reduction in the pH of the blood might be more marked. The CO_2 was administered from bags of 25 c.f. capacity, the gas mixture being analysed at the beginning and at the end of each bag. The bags were sufficiently impermeable to CO_2 and these two analyses rarely differed appreciably. Carbon dioxide was administered in different concentrations in the several experiments which fall conveniently into three groups—those in which 5–6 p.c. of CO_2, 7–8 p.c. and 9–10 p.c. CO_2 respectively, were given. It will be sufficient to quote one experiment from each group as representative of the results obtained.

In Exp. 3 whilst the preparation was ventilated with air the concentration of lactic acid was increased slightly. Administration of 5·4 p.c. CO_2 caused an increase in the CO_2 content of the blood and reduced the pH. The increase in lactic acid was definitely arrested. The rate of disappearance of the blood sugar did not seem markedly affected by the CO_2. The oxygen saturation of the blood was good throughout.

In Exp. 4 the heart rate fell to 80 beats a minute upon the first administration of CO_2 but it soon regained its normal rate. It will be seen that the fall in the pH was followed by a diminution in the concentration of lactic acid. Unfortunately the bag containing the CO_2 mixture became exhausted ten minutes before the last blood sample was drawn. This explains the high pH and low CO_2 content recorded for this sample. It would appear, however, that the short interval of this alkalæmia was insufficient to be reflected in the lactic acid concentration which was evidently at the value determined by the previous $1\frac{1}{2}$ hours under the influence of CO_2. The reduction of the lactic acid concentration was slow, being reduced by one-half in $2\frac{1}{2}$ hours. The sugar disappeared from the blood during the course of the experiment

Exp. 3. Effect of 5·4 p.c. CO_2. Blood-pressure: 85. Output: 350 c.c. Heart rate: 110. Temp.: 36·5° C.

Time	*p*H	CO_2 content	Lactic acid	Sugar	Oxygen saturation	Hb
0.20	7·78	18	39·0	220	—	100
0.40	7·74	16	47·9	167	95	100
0.50	Air with 5·4 p.c. CO_2 for the rest of exp.					
0.53	7·62	24	—	—	—	—
0.56	7·53	30	—	—	—	—
1.00	7·44	40	50·2	142	93	102
2.30	7·42	45	43·0	07	—	102
4.00	7·42	45	40·0	87	96	104

Exp. 4. Effect of 7·2 p.c. CO_2. Blood-pressure: 95. Output: 320 c.c. Heart rate: 103. Temp.: 36° C.

							Blood chlorides	Serum chlorides	Serum solids
1.15	7·74	17	64·0	72	94	100	570	720	7·24
2.00	7·70	0	76·2	39	95	101	570	720	7·28
2.07	Air with 7·2 p.c. CO_2								
2.30	7·38	56	72·0	15	93·5	102	570	705	7·35
3.00	7·34	58	60·9	0	96	104	560	695	7·48
4.20	Air without addition of CO_2								
4.30	7·83	11	38·0	0	89	105	555	710	7·56

Exp. 5. Effect of 9·4 p.c. CO_2. Blood-pressure: 90. Output: 350 c.c. Temp.: 36·5–37° C. Heart rate: 130–140.

1.10	7·77	11	70·5	—	—	100	580	780	7·63
1.16	Air with 9·4 p.c. CO_2 for the rest of exp.								
1.35	7·30	45	23·3	—	—	101	580	740	7·67
2.35	7·22	60	10·6	—	—	101	575	740	7·81
3.55	7·22	60	—	—	—	102	570	740	7·90
5.00	7·22	60	10·6	—	—	104	570	745	7·92

but, as has been stated, ample evidence has been obtained that lactic acid continues to accumulate in the blood of a normal heart-lung preparation long after the blood is sugar-free. The loss of lactic acid in this experiment cannot, therefore, be explained by the disappearance of the sugar.

A comparison of Exps. 3–5 shows that the administration of CO_2 in increasing concentrations of 5·4, 7·2 and 9·4 p.c. reduced the *p*H of the blood respectively to 7·4, 7·34 and 7·22 with corresponding increases in the CO_2 tension. In the same sense the effect on the lactic acid was increasingly marked. A fall of *p*H from 7·78 to 7·40 was followed by complete arrest of the lactic acid accumulation and a tendency towards reduction, a fall of *p*H from 7·77 to 7·34 effected steady but slow reduction in the lactic acid by 50 p.c. in 2½ hours, whilst a shift of *p*H from 7·77 to 7·30 reduced the lactic acid by a third in 20 minutes, and a further change to 7·22 brought the concentration down to one-seventh of its initial value. In no case was there sufficient change in the oxygen saturation of the blood to suggest an explanation for these results.

of the arterial blood are of little value. Analyses of the mixed venous blood would have been more appropriate. However, two experiments which were performed exhibited the same changes in lactic acid concentration as demonstrated in the heart-lung preparation. In Exp. 8 it will be seen that the concentration of the lactic acid did not diminish until the *p*H of the blood was reduced by administration of CO_2. When the CO_2 was removed the *p*H rose again and the lactic acid returned. Although there was no evidence that the CO_2 improved the oxygenation of the tissues by improving the circulation, the fact of the deficient circulation does detract from the value of the observations.

Exp. 8. Dog. Brain and spinal cord destroyed under C.E. anæsthesia. Blood samples collected from femoral artery.

Time	*p*H	CO_2	Lactic acid	Oxygen saturation of arterial blood	Hb
0.00	Spinal cord cut				
0.20	7·52	46	166	92	100
0.22	Excessive respiration with air				
1.32	7·65	17	175	93·6	98
1.35	Excessive respiration with air containing 5·2 p.c. CO_2				
2.45	7·22	74	117	91·8	100
2.50	Excessive respiration with air				
3.15	7·68	13	165	94·3	103

Exp. 9. Heart-lung preparation.

0.40	7·75	12	53	94	100
0.40	Air with 8·2 p.c. CO_2				
1.20	7·34	59	31	95	100
1.25	Gas mixtures containing 8·2 p.c. CO_2 and 3·9 p.c. O_2				
3.20	7·30	67	13	41	101
3.25	Air without CO_2				
4.00	7·78	9	49	95·5	103

The effect of anoxæmia. Much stress has been laid on the fact that the oxygen saturation of the blood in these experiments has been reasonably constant. Experiments now in progress have for their object a study of the relation of the oxygen saturation of the blood to the lactic acid concentration. Whilst these are as yet incomplete we believe that we are in a position to state that in the heart-lung preparation considerable reductions in the oxygen saturation can be tolerated without increase in lactic acid, provided that the *p*H of the blood be kept down by the administration of CO_2. Exp. 9 may be quoted.

After 40 minutes of ventilation with air the *p*H was found to be 7·75 with a lactic acid content of 53 mgms. p.c. Administration of 8 p.c. CO_2 reduced the *p*H to 7·34 and the lactic acid to 37 mgms. p.c., and when ventilation was continued with an 8·2 p.c. CO_2 mixture containing only 3·9 p.c. oxygen, the *p*H moved slightly further to the acid

side and the lactic acid concentration continued to fall in spite of an arterial oxygen saturation of only 41 p.c. When the latter was increased to normal and, at the same time, the CO_2 removed, the lactic acid concentration increased nearly fourfold.

In a discussion of changes in oxygen saturation of the blood in relation to anoxæmia not only has the amount of oxygen carried by the blood to be considered but also the oxygen tension. The latter was not determined directly but may be calculated from Hill's equation:

$$\frac{y}{100-y}=kx^n,$$

where the constant k may be deduced for a given blood sample from the equation:

$$\frac{1}{k}=a\,cH\,10^{9};$$

n was taken as 2·2 and a as 360 (11). Now an application of these calculations to Exp. 5 shows that whereas the oxygen saturation was about 95 p.c. throughout, the shift in pH from 7·77 to 7·22 would involve an increase in oxygen tension of nearly 100 p.c. That this increase was not the explanation of the diminution in lactic acid is apparent by a consideration of Exps. 2 and 9. In the former there was a steady fall in pH associated with a constant oxygen saturation and therefore a steady rise in oxygen tension. In spite of this the lactic acid continuously accumulated. In Exp. 9 the oxygen saturation was reduced artificially from 95 to 41 p.c. without appreciable change in pH. The oxygen tensions corresponding to these two conditions were calculated to be 90 and 22 mm. Hg respectively. Nevertheless the lactic acid diminished considerably. On the ground of all these considerations it would seem that the factor controlling changes in the lactic acid concentration of the blood in these experiments was not the oxygen tension of the blood.

Experiments with blood in vitro. It was of importance to determine if the phenomenon of glycolysis in blood *in vitro* bore any relation to the experimental results under discussion. Is this regulatory mechanism resident in the blood or dependent on the presence of tissues? Defibrinated dogs' blood was incubated at 37° C. in tonometers in which equilibration was effected with varying mixtures of air and CO_2. The changes in the blood sugar and in the lactic acid were followed.

Exp. 10.	pH	CO_2 content	Sugar	Lactic acid
Blood at beginning ...	7·72	29	164	33
After 4 hrs. with air ...	7·66	20	74	80
„ „ 19 p.c. CO_2	7·10	80	109	75

5. The mechanism regulating the lactic acid concentration of the blood forms with the primary and secondary buffering systems a third line of defence against excessive changes in the reaction of the blood.

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ERRATA

Vol. 57. p. vii. In List of Authors. After "Wigglesworth and others" *for* "Antidromic action" *read* "Insulin and blood phosphate."

Vol. 58. No. 1. p. 31. In Table III, *for* "c.c. oxygen grm./min." *read* "c.c. oxygen grm./hrs."

„ „ p. 67. *For* "Euglein" *read* "Engling."

CHELONIAN RESPIRATION (TORTOISE).

By THOMAS LUMSDEN, M.D.

(From the Department of Experimental Pathology, Lister Institute.)

In a previous communication (1) it was mentioned that in certain reptiles (*e.g.* tortoise, turtle, crocodile, alligator) the respiration is normally of apneustic type, *i.e.* consists of a series of prolonged inspirations. Since this fact has a possible bearing on the evolution of mammalian respiration and more or less elucidates the occurrence of apneustic breathing in mammals, after elimination of the highest (pneumotaxic) respiratory centre, further investigations were made in tortoises with the results herein reported.

Methods. A respiratory tracing was obtained by slipping a fairly stout but pliable rubber tube over the head of the tortoise. Through this tube the animal breathed various gases, from a large bottle with which a manometer and a recording tambour were connected. The effects of stimulation and section of the vagi and brain stem, and of the application of heat and cold, were observed. Anæsthesia was obtained by injection of chloral (·1 to ·5 gm.) or by freezing the cerebrum by prolonged external application of ethyl chloride with subsequent decerebration. In tortoises as in mammals, however, chloral so powerfully and so indiscriminately affects the activity of the respiratory centres, as to render the results obtained unsuitable for the study of normal breathing. Decerebration allows of much more reliable conclusions.

Tortoises resist attempts to chloroform them by simply refusing to breathe for 2–3 hours at a stretch. It is, however, possible ultimately to obtain anæsthesia by means of chloroform especially if the tortoise has been breathing N_2 for some time previously, or if the temperature of the animal is raised to about 30° C. Chloroform vapour may be pumped into the animal's lungs by positive pressure. The method is not very satisfactory, for it is difficult to know when to stop, and even a slight overdose means death from cardiac failure.

Normal respiration. John Hunter (2) concluded that inspiration was effected "by the muscles of the extremities moving their respective bones in an inverted order: instead of moving the extremity, the extremity becomes the fixed point: the bones answering to the clavicles are moved

forward and the bones of the pelvis are pushed against the inside of the breast-bone so that the whole bone is pushed out." Owen(3) stated that "The lungs of the chelonia appear to be filled with air chiefly by acts of deglutition." Neither of these views correctly represents the facts. Respiratory movements causing only slightly diminished interchange of air, still occur even if the limbs are held entirely within the carapace, or in the completely extended position, and careful observation makes it clear that the contraction of the parietal muscles of the body cavity causing expiration, are powerful and independent of the limb muscles. With regard to the throat movements, which Owen considered respiratory and in the nature of deglutition, Hunter had previously and correctly concluded that these were not inspiratory. They go on frequently during the respiratory pause, even if the animal is under water, and they are not accompanied by either intake or output of air and so do not affect the respiratory tracing, though they may have some effect in altering the distribution of the air already inspired.

The facts are as follows. The prolonged inspiratory pause is terminated by a rapid profound expiration. A deep inspiration immediately follows, and the breath is retained for as a rule a half to five minutes. Inspiration is effected by muscles which rotate the scapula and clavicles inwards and forwards, but the fixed point is the shell and not the extremity; this movement favours, though it does not necessitate, protrusion of the head and fore limbs and markedly enlarges the front part of the body cavity. In a somewhat similar way the pelvis is drawn bodily backwards and downwards, carrying with it the hind limbs.

During normal expiration the tone of the inspiratory muscles relaxes and the air is expelled by contraction of the flat subcutaneous muscles of the body wall. If very deep expiration is called for (*e.g.* while breathing 30 p.c. CO_2) accessory muscles of expiration rotate the shoulder girdle and pelvis in the reverse of the inspiratory directions. Such forced expiratory movements are accompanied by retraction of the head and limbs, and may be repeated two or three times between successive apneuses (Fig. 1).

The lungs being fixed all along their dorsal surface to the inside of the carapace, it follows that while in the natural position, the weight of the other viscera dragging on the front of the lungs to which they are attached by peritoneal folds must tend towards inspiration. Since, however, the animal can breathe as fully while resting on its back, active muscular action must be the main inspiratory factor. During the inspiratory pause a strong negative pressure, of an intensity varying

in different apneuses, is maintained in the whole recording apparatus. Thus when excess of CO_2 is administered, not only is expiration increased in depth, but the inspiratory negative pressure is also markedly exaggerated even when the animal is placed on its back. This could not occur if inspiration were passive in origin. In the dead tortoise, turning the animal on its back or pushing the limbs and head inwards produces, of course, expulsion of air, and the reverse conditions cause indrawing of air; but when alive the animal can move its head, tail, and limbs freely in all directions without producing either expiratory or inspiratory air currents.

Effect of section of the brain stem at various levels. By carefully clipping away the calavarium it is possible to expose the whole of the brain stem, which may then be divided at any desired level. The tissues are very delicate and must be cautiously dealt with if consistent results are to be obtained.

Section down to just about the level of the middle of the fourth ventricle leaves respiration unaltered in type.

Section below this level destroys first the power to maintain inspiratory tonus, and expiratory movements alone occur.

Section just above the apex of the calamus scriptorius abolishes expiratory manifestations and gasping may occur alone, but bleeding is so free that respiration very soon ceases altogether. The animal is now purely spinal and its limb reflexes are much more rapid and powerful than usual. The heart continues to beat for some hours, but all spontaneous and respiratory effects cease permanently. I never saw any evidence of the existence of spinal respiratory centres, though the movements of the limbs when reflexly elicited do produce some interchange of air. It is noteworthy that gasping is much less persistent and less commonly seen in tortoises than in mammals. The reason of this may be that a considerable amount of inspiration is maintained by gravity; hence expiration becomes, when the tortoise is *in extremis*, more effective than inspiration. Thus, in the evolution of this animal, gasping, being useless, may have more or less failed to survive.

Effects of anæsthetics. When a full hypodermic dose of chloral is given, *e.g.* $\frac{1}{2}$ to 1 gm. to a large tortoise, the breathing gradually becomes very shallow and regular in rhythm. Each breath is slowly effected but the frequency increases say to 6 per min. Sometimes it becomes intermittent, *i.e.* of periodic type, a dozen slow breaths may be taken, and then a pause of several minutes occurs before the next series of breaths. The animal is in a condition very much like hibernation.

Voluntary movements cease and reflex movements are less easily evoked and less actively performed than normally. Chloroform has a rather different effect, it affects the centres more clearly in order from above down. As the anæsthesia gets deeper, first the power to maintain inspiratory tonus disappears, then expiratory movements cease and finally pure, though slow, gasping movements may occur. Recovery takes place in the reverse order (Fig. 1).

Peripheral nervous influences. Strong vagal stimulation during either active inspiratory or expiratory movements inhibits them. There is not, however, anything very distinctive in this, for strong stimulation

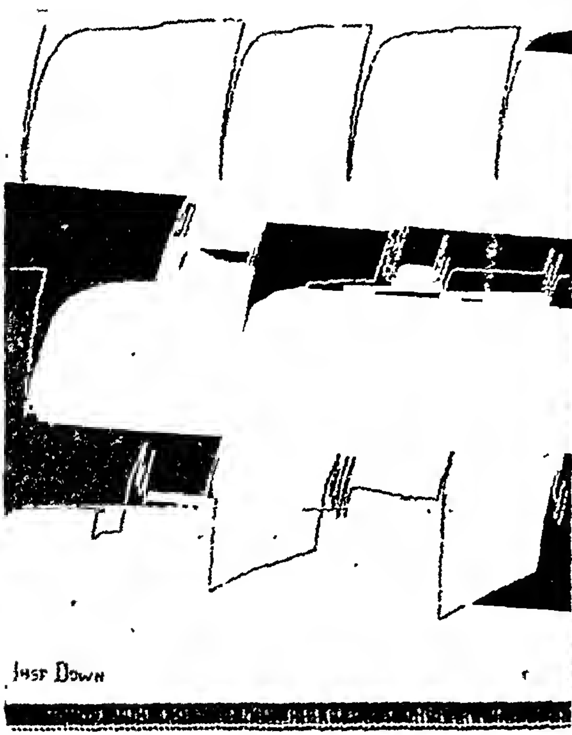

Fig. 1.

Fig. 2.

Figs. 1 and 2. Inspiration down. Time tracing, 5 secs. Parallel lines = base line of atmospheric pressure.

Fig. 1. Respiratory recovery after overdose of anæsthetic. Top tracing, gasping. Middle tracing six minutes later, expiration recommences. Lowest tracing after 17 minutes, revival of apneusis.

Fig. 2. Top tracing, normal rapid breathing. Middle, after one hour on 30 p.c. CO_2. Lowest, after one hour on pure N_2.

of other sensory nerves has a similar, though somewhat less pronounced effect. The vagi appear to have no tonic respiratory influence, for vagotomy does not affect either the rate or the depth of breathing. Stimulation of the fifth cranial nerves produces instant retraction of the head accompanied by active and complete expiration.

Effect of $+ CO_2$ *and* $- O_2$. If the anæsthetised or decerebrate animal breathes pure N_2 the respiration becomes gradually more rapid. After an hour or so the maximal speed of say six breaths per minute may be attained; as exhaustion supervenes the breathing becomes slower again but may continue for three hours or more to be of normal type. Thereafter expiration first becomes spasmodic and almost convulsive while inspiratory tonus no longer occurs: at this stage inspiration is mainly passive. Later still in some cases expiration ceases and inspiratory gasps alone occur, the mouth opening widely with each gasp. During dyspnœa due to N_2 the respiratory movements, though more frequent, are but little deeper than usual (Fig. 2).

Respiration of an atmosphere composed of CO_2 20–30 p.c., O_2 30–40 p.c. and N_2 30–50 p.c., causes marked increase in the amplitude of the respiration and the frequency is slightly increased. Expiration becomes very active and spasmodic and inspiration is much deeper than normal. I have never seen any harmful effects even after three or four hours of such respiration (Fig. 2).

Influence of heat and cold. If either of these is excessive (over 40° C. or freezing) and suddenly applied, incoordinated reflex inspiratory and expiratory movements may occur instantly. But heat up to 40° C. gradually applied and cold down to 10° C. produce interesting and constant effects. If the tortoise is partially immersed in a bath of warm water at 37° C. it very gradually breathes quicker and quicker as its body temperature rises, and if it is now cooled with cold water the respiration becomes slow again or stops altogether. This is not, as might have been surmised, merely because the general metabolism has increased or diminished in activity proportionately with the body temperature. I found that exactly the same respiratory effects can be produced by varying the temperature of the blood going to the head by continually warming or cooling the neck; although the general body (rectal) temperature remains unaltered or is even made to vary inversely with the neck temperature.

These effects are, perhaps, most strikingly seen in a chloralised tortoise breathing, as is usual, very feebly and regularly. Warming the neck to 37° C. produces (only after about three minutes) very marked

increase in the amplitude of breathing with some increase also in frequency. If now the neck is cooled with cold tap water, the breathing slows and lessens, so that after two to four minutes it ceases altogether for 10 or 12 minutes. If the cooling is now stopped the respiration resumes the usual chloralised type. This experiment can be repeated as often as is desired and the results can be predicted with absolute certainty (Fig. 3).

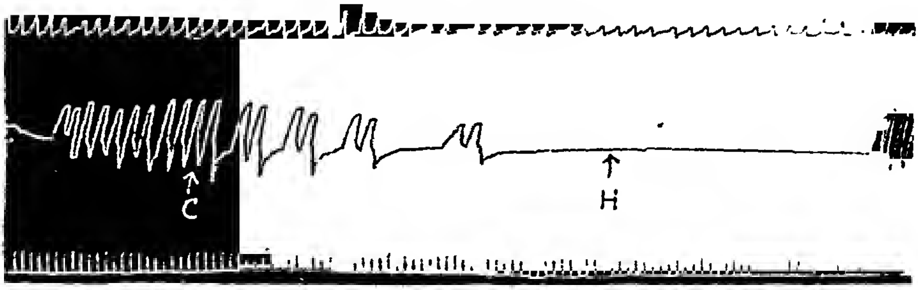

Fig. 3. Upper tracing, respiration after large dose of chloral. Lower tracing from same animal, effect of heating and cooling neck. At *C* cold was applied. Two minutes before tracing begins and at *H* heat was applied. Expiration up. Time tracing, 5 secs.

So marked is this regulation with heat and cold that it overcomes any effects due to variations in the gas inspired. Thus a tortoise breathing CO_2 30 p.c., N_2 70 p.c., breathes much slower if its neck is cooled than the same tortoise breathing O_2 50 p.c., N_2 50 p.c., when its neck is kept warmed to 37° C. In a particular instance a tortoise on oxygen, breathed normally once in every three minutes, when its neck was warmed with water at 37° C., its respirations became two per minute. The oxygen was now replaced by CO_2 30 p.c., N_2 70 p.c., the breathing increased to three per minute. The neck was now cooled to 10° C., the breathing gradually became shallower and slower till only one breath was taken in four minutes. During the whole experiment the rectal temperature remained 18° C.

From this observation it appears that warming the blood going to the head acts directly on the respiratory centres and not by increasing metabolism, for here the rate of breathing varies inversely as the variation which should have been produced by the gases inspired and which were such as increase or diminution of metabolism would produce. It would seem then that the respiration in tortoises is regulated more in accordance with the heat of the blood supplying its brain than in accordance with the nature of its metabolic needs or of the gas it inspires. Even when at the height of dyspnœa due to breathing – O_2 or + CO_2 the respiration

can be markedly lessened and slowed or even stopped by cooling the neck. The cause of the respiratory increase due to heat is not the warmth of the air entering the lungs, for this may be warmed to 45° C., yet if the neck is cooled respiration will be slow.

Remarks. The respiratory rhythm in a tortoise appears to depend primarily upon the temperature of the blood reaching the respiratory centres. Under normal conditions this will vary with the general body temperature and will be proportional to the atmospheric temperature.

If the temperature is constant the respiration is regulated chiefly by the amount of CO_2 in the blood which will vary with the activity of the animal and of its metabolism. The temperature is the coarse and CO_2 the fine adjustment.

The time (2–4 minutes) which elapses before heat or cold influences the respiration is much longer than is required for a nervous reflex from the skin of the neck or head; it is much shorter than the time required for metabolic needs to become effective in this animal, as is evidenced by its slow response to excess of CO_2 or lack of O_2.

Since heating or cooling the inspired air does not appreciably alter the respiratory rhythm it may be concluded that the alteration of breathing produced by heating or cooling the neck is due to changes in the temperature of the cells composing the respiratory centres.

Either local cold or warmth transmitted through the skull, or variation in the temperature of the blood reaching the brain stem, appears to be an adequate stimulus in this direction, but the respiratory centres are much more readily influenced by way of the blood than by applying heat or cold to the skull, for if the neck is warmed while the head is cooled the respiration becomes rapid, conversely cooling the neck and warming the head lessens the breathing or temporarily stops it.

The actual event determining the respiratory rate is the incidence of expiration, which occurs when the excitability of the expiratory centre has been raised by the CO_2 passing through it, to a point at which it overcomes the existing inspiratory tonus. The instant expiration has taken place, the excitability of the expiratory centre is at its lowest and apneusis is resumed.

The amplitude of the respiratory interchange depends in the tortoise as in mammals on the amount of CO_2 in the blood stimulating more or less intensely the apneustic and expiratory centres. Lack of O_2 is much less effective than excess of CO_2 in this direction.

In the evolutional scale reptilian respiration represents a very fair half-way stage between the fish gasping for breath when taken out of

the water, and the highly developed mammalian respiratory mechanism. Between the gasping fish and the tortoise intermediate stages may be sought in amphibians and in the mud fishes.

Conclusions.

1. The respiratory centres found in the tortoise are the gasping centre at the apex of the calamus scriptorius, the expiratory centre just above this and the apneustic centre about the level of the middle of the fourth ventricle. There is no evidence of the existence of a pneumotaxic centre in this animal.

2. The vagi do not appear to have any tonic regulating effect on the respiration in tortoises.

3. The respiratory needs are so slight that under certain conditions a single inspiration may last for two to three hours.

4. The respiratory rhythm is more powerfully affected by variations in the temperature of the cells composing the respiratory centres than by variations, in the activity of the general metabolic processes, or in the gases respired. When, however, the temperature is constant, the amount of CO_2 in the blood appears to be the chief factor in the regulation of normal respiration.

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THE MUTUAL INFLUENCE OF SECRETORY STIMULI IN THE SUBMAXILLARY GLAND OF THE CAT.

By E. E. GOLDENBERG.

(*From the Physiological Laboratory, University of Odessa.*)

The importance of the meaning of the phenomenon established and called by Langley(1) "augmented secretion" has incited me to investigate more accurately the mutual influence of any two secretory effects and also of some of the vasomotor effects in the submaxillary gland of the cat.

Methods. Cats were anæsthetised with ether and chloroform and received an injection of 1 to 2 c.c. of 1 p.c. solution of hydrochloride of morphia. A cannula, connected with a graduated tubing, was inserted into the duct of the submaxillary gland. The chordo-lingual nerve and sympathetic in the neck on the corresponding side were cut and placed on the fixed electrodes, connected with two induction coils. To measure the blood flow of the gland all the veins around the gland were tied. The blood drops fell from the incision made in the external jugular vein, the central end of which was tied. The duration of each stimulation was usually 15 seconds. The secretion and the blood flow were also measured, usually during each 15 seconds.

Description of protocols and figures. In the protocols of the experiments and in the figures the quantity of saliva secreted is given in divisions of the graduated tube connected with the duct. In the protocols the numbers in thick type show the periods of stimulation. All curves are constructed so that not only the immediate effect of each stimulation was taken into account but the after effect also.

Influence of two successive stimulations of the gland itself. The surface of the gland was laid open and the electrodes were applied to this part. The figures of Exp. 1 show that the effect of the second stimulation applied 30 seconds after the first is several times stronger than the effect of the first.

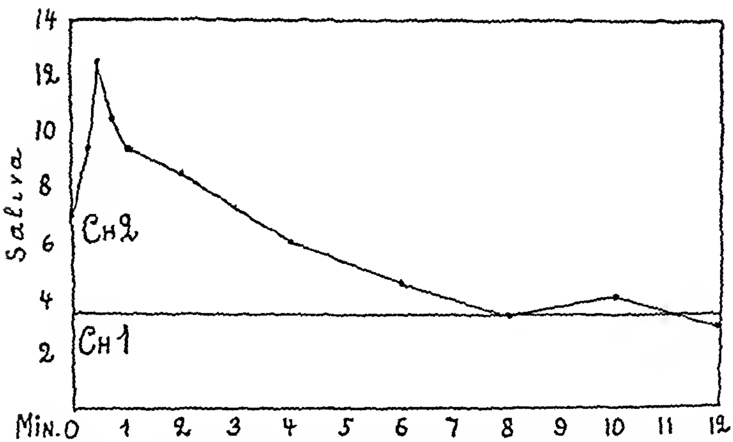
Exp. 1. Gland stimulated. Coil at 5 cm.

4.25 p.m.	Saliva secreted in each 15 secs.	**1**,	2½,	0,	5,	4½,	0,	5,	5½,	½	0
5.20	Saliva each 15 secs.	**2½**,	5.	½,	**11**,	5,	1,	0			
5.35	Saliva each 15 secs.	**1½**,	3,	0,	**11**,	4.	½,	½,	0		

The stimulation of chorda tympani after chorda tympani. Two successive stimulations of the chorda were performed, the strength of the stimulation and the part of the nerve stimulated being the same. The intervals between two stimulations varied from 0 to 14 minutes. Each pair of these stimulations was divided from the following pair by an interval lasting from 12 to 15 minutes. As an example I give here Exp. 2. The arrangement of all other experiments was the same. Exp. 2 (Fig. 1) shows that the second stimulation reached its maximum effect after an interval of 30 seconds. In from 8 to 10 minutes both stimulations gave practically the same results.

Exp. 2. Chorda stimulated. Coil = 18 cm. Saliva flow noted each 15 secs. In this, as in the other experiments, the periods of stimulation are in thick type.

Interval	
0 secs.	1½, 5, 2, 0 divisions of graduated tubing
15 ,,	2½, 2, 7½, 1½, ½, 0
30 ,,	1½, 2, 0, 10, 2½, 0
45 ,,	2, 2, 0, 0, 8, 2, ½, 0
1 min.	2½, 1½, 0, 0, 0, 7½, 2, 0
2 mins.	2, 1½, 0 ... 6½, 2, 0
4 ,,	2, 1½, 0 ... 4, 2, 0
6 ,,	1½, 2½, 0 ... 2½, 2, 0
8 ,,	2½, 1½, 0 ... 2, 1½, 0
10 ,,	after the last stimulation 2, 2, 0
12 ,,	,, ,, 1½, 1½, 0
14 ,,	,, ,, 1½, 1, 0



ig. 1. Stimulation of chorda after chorda. *Ch.* 1 = average quantity of saliva secreted as the result of the first stimulation. *Ch.* 2 = total quantity of saliva secreted as the result of each second stimulation.

The influence of two successive stimulations of chorda upon the blood ow through the vessels of the gland is only seen if the interval between two stimulations does not exceed one minute. The blood flow during the

second stimulation, as a rule, is greater than during the first stimulation. But the quantity of blood flowing through the vessels in the following 15 seconds equalises the effect of both stimulations (Exp. 3).

Exp. 3. *Blood flow through the vessels of the gland. Chorda stimulated. Coil at* 16 cm.

Interval	
0 sec.	1, 0, 9, 18, 13, 8, 6, 5, 3, 5, 5, 6, 4, 4, 1, 1 drops of blood
15 „	1, 1, 6, 10, 6, 3, 3, 2, 3, 3, 3, 3, 3, 1, 1, 1
30 „	1, 1, 1, 8, 17, 5, 14, 11, 2, 2, 2, 2, 2, 1, 2, 2, 2, 1, 1, 1
1 min.	0, 1, 0, 5, 16, 4, 2, 1, 9, 13, 4, 1, 2, 1, 0, 1
3 „	1, 1, 7, 9, 3, 2, 2, 2, 2, 2, 2, 1, 2, 1, 1, 5, 9, 2, 1, 3, 2, 2, 2, 2, 2, 1, 1
5 „	1, 1, 2, 6, 13, 3, 2, 2, 2 ... 1, 1, 5, 12, 3, 2, 2, 3, 3, 2, 2, 2, 1, 1

Thus in the blood vessels the conjoint effect of two stimulations differs in a certain degree from that observed in the secretory cells.

The stimulation of sympathetic after the sympathetic. In cases of the sympathetic nerve supraliminal or subliminal stimulations were always used. The second stimulation was more effective if the interval dividing it from the first did not exceed two minutes. The maximum effect is observed after an interval lasting 15 seconds (Fig. 2).

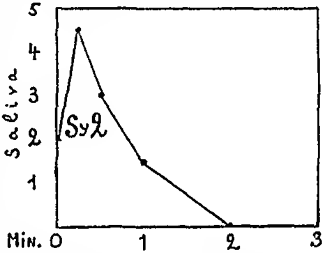


Fig. 2. Sti[illegible] sympath[illegible] secreted [illegible] sympathetic is almost nil. Sy. 2 — total quantity of saliva secreted as the result of every second stimulation of the nerve.

As to the blood flow through the gland, we can see that successive stimulations of sympathetic nerve influence one another if the interval between them does not exceed 45 to 60 seconds (Exp. 4).

Exp. 4. Blood flow through the blood vessels of the gland. Sympathetic stimulated. Coil = 11 cm.

Interval											
0 secs.	1,	1,	2,	2,	1						
15 „	1,	1,	4,	1,	2,	2,	2,	1			
30 „	1,	0,	3,	2,	1,	1,	1				
1 min.	1,	0,	2,	3,	1,	1,	0,	1,	3,	1,	1

A more pronounced successive inhibition of the blood flow after the second stimulation is to be seen here. More rarely, however, I noticed under the same conditions an increased blood flow through the gland.

Stimulation of sympathetic after chorda. This is the case of Langley's "augmented secretion." The maximum secretory effect was obtained here as a result of immediate stimulation of the sympathetic after the

stimulation of chorda (interval = 0 sec.). This can be explained by summation of the sympathetic effect with the after effect of the chorda. Except this, the maximum secretory effect is reached here also when the interval between two successive stimulations is equal to between 30 secs. to 1 min. (Fig. 3).

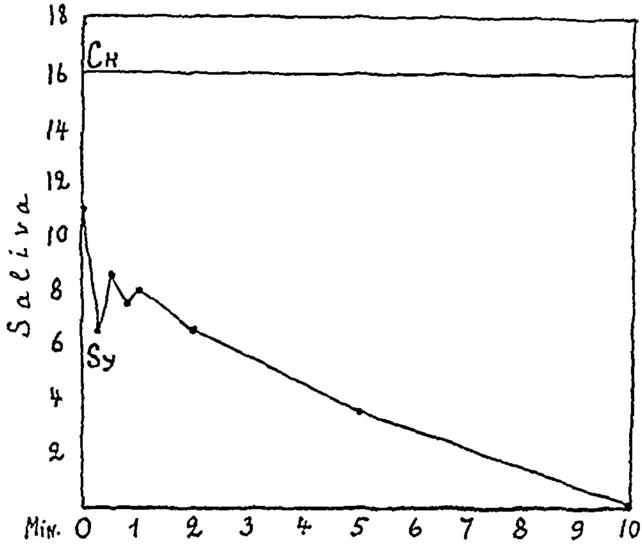


Fig. 3. Stimulation of sympathetic after chorda. Ch. = average quantity of saliva secreted by stimulating the chorda tympani. Sy. = total quantity of saliva secreted by each stimulation of sympathetic.

Stimulation of chorda after the sympathetic. The stimulations applied to the sympathetic in these cases were near to the threshold of excitation. Successive weak stimulation of the chorda tympani gave an increased secretion, which reached its maximum in 30 to 45 seconds after the stimulation of sympathetic nerve (Fig. 4).

I also observed the increased effect of two simultaneous subliminal stimulations of chorda and sympathetic. These observations complete the data of Langley (2) who has seen the augmented secretion applying supraliminal stimulations to the chorda and subliminal stimulation to the sympathetic.

The length of the interval of the summation. There is a notable difference in the duration of the influence which one secretory nerve produces upon another. If the chorda tympani is stimulated first the augmented secretion can be observed for a period of 8–10 minutes. In cases of primary stimulation of the sympathetic the increased effect can be obtained only for 2–2½ minutes. Two explanations can be given of this

phenomenon: (1) the different action of parasympathetic and sympathetic innervation or (2) the different degree of excitation of the gland during

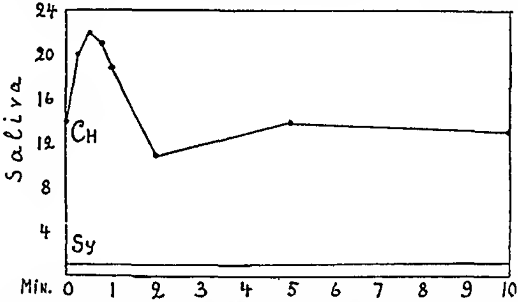


Fig. 4. Stimulation of chorda tympani after the sympathetic. Sy. = average quantity of secreted saliva by stimulating the sympathetic. Ch. = total quantity of saliva secreted by each stimulation of chorda.

the first stimulation. The following experiments show that the second supposition sufficiently well explains the phenomena observed. As it was impossible to get a sufficiently large secretion by strong stimulation of the sympathetic, I used two successive weak stimulations of the chorda.

In Exp. 5 the interval between the first and the second stimulation of the chorda was three minutes. But the strength of the first stimulation varied. Corresponding to this, the secretory effect of the second stimulation varied also.

Exp. 5.

Coil	18 cm.	19 cm.	20 cm.	21 cm.
First stimulation of chorda	3	$1\frac{1}{2}$	0	0
Second stimulation of chorda after 3 mins.	$7\frac{1}{2}$	5	$\frac{1}{2}$	0

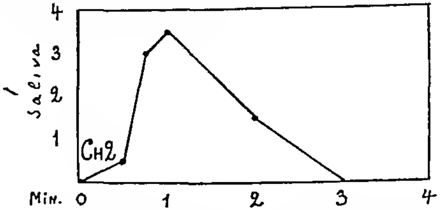


Fig. 5. Stimulation of chorda after chorda. Ch. 2 = total quantity of saliva secreted by every second stimulation of the nerve. The effect of the first stimulation was very small.

SOME PECULIARITIES OF THE SYMPATHETIC INNERVATION OF THE SUBMAXILLARY GLAND OF THE CAT. By P. M. JURIST AND B. A. RABINOVICH.

(*From the Physiological Laboratory, University of Odessa.*)

The comparative study of the innervation of the salivary glands in different animals may throw a light on the meaning of the double nerve supply to these organs. Sinelnikov(1) has shown that the degeneration of the preganglionic secretory and vasomotor fibres of the sympathetic nerves to the submaxillary gland in the dog takes place in varying times. The vasomotor fibres lose their excitability in from three to four days after their section; the secretory fibres are still active up to the sixth day. In young dogs and puppies the secretory fibres become completely inactive in from three to four days after section.

At the suggestion of Prof. B. P. Babkin we repeated these experiments in cats in respect to the great difference in the action of the sympathetic nerve upon the submaxillary gland in both animals. The only indication we have found about the time of degeneration (three days) of the secretory sympathetic fibres to the submaxillary gland is in the cat in Bradford's(2) paper.

Methods. The aseptic operation of section of the cat's sympathetic nerve in the neck was performed from 27 to 95 hours before the experiment. During the experiment the animal received either chloroform and ether and a subcutaneous injection of 1·0 to 1·5 c.c. of 1 p.c. solution of hydrochloride of morphia, or it was decerebrated. The chorda tympani was cut, a cannula introduced into the duct of the submaxillary gland and the blood vessels of the gland prepared in the usual manner for the observation of the blood flow. The sympathetic was stimulated by an induction current several times before and after the stimulation of the chorda tympani.

From the table it can be seen that complete inactivity of both secretory and vasomotor fibres of the sympathetic occurs in from 38 to 50 hours after the section of the nerve in the neck. Between the 41st and 48th hours there is a considerable weakening of both effects, but

the vasomotor fibres apparently lose their excitability a little earlier than the secretory fibres.

Exp.	Time after the section of the sympathetic	Secretory fibres	Vasomotor fibres
June	95 hours	Inactive	Unexamined
"	53 "	"	"
Dec	52 "	"	Inactive
"	51 "	"	"
June	50 "	"	"
Nov.	48 "	"	"
"	47 "	Feebly active	"
Feb.	45 "	"	Feebly active
Oct.	41 "	Active	Inactive
"	32 "	"	Active
June	27 "	"	Unexamined

Thus in the cat the time of the loss of the excitability of both secretory and vasomotor preganglionic fibres is approximately the same. In the dog these times are different; after the section of the nerve the vasomotor fibres lose their excitability from 36 to 48 hours earlier than the secretory.

Summary.

The excitability of both sympathetic secretory and vasomotor fibres of the submaxillary gland of the cat after the section of the nerve in the neck is lost in from 41 to 48 hours.

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SECRETION AS A FACTOR IN ELIMINATION BY THE BIRD'S KIDNEY. By E. B. MAYRS.

THE experiments on which the absorption theory of urinary secretion is based have been carried out for the most part on the amphibian and mammalian kidney. In the bird, however, absorption of fluid is largely relegated to the cloaca, and it is of interest to determine whether any modification of the renal process is associated with this peculiarity.

The kidney of the fowl is an elongated organ attached to the lower ribs; it is partially lobulated, and the ureter is formed by the junction of several tributaries embedded in the substance of the lobules. Microscopic examination, however, does not reveal any obvious structural departure from the mammalian type. The ureters are thick-walled ducts which open into the cloaca just above its sphincter. The normal urine is semi-solid in consistence, owing to a deposit of uric acid or urates; it contains a large amount of mucous material, and is usually mixed with fæces. But when urine is collected from the ureters without having had access to the cloaca it is found to be fluid, though often somewhat slimy in character. Excretion is generally rapid, and the liquid is sometimes quite transparent but frequently contains flakes of deposit. The flow of urine is presumably reduced by anæsthesia, with consequent increased absorption of water, and it is possible that under normal conditions uric acid is all in solution when it leaves the kidney. In any case, a great deal of water must be re-absorbed from the cloaca.

Minkowski(1) has obtained evidence that the uric acid of birds is formed for the most part from ammonium lactate, which is probably an end-derivative of protein catabolism. Milroy(2) found more recently that when acid is given to fowls the output of ammonia is increased at the expense of uric acid. In the metabolism of the bird, therefore, uric acid occupies a position analogous to that of urea in mammals, as a waste product derived chiefly from exogenous protein; and the need for continuous excretion of this substance is evident. The bird, however, is at a disadvantage, since its chief waste product is relatively insoluble; and the curious excretory arrangement which it has developed may have been designed to overcome this difficulty. Whether uric acid is

eliminated by secretion or by glomerular filtration, it is unlikely to exist in the solid form in any cells of the kidney. Uric acid crystals have been observed in the tubule cells of the bird by v. Wittich(3). A post-mortem change in the reaction of the cell fluid might, however, have caused precipitation of uric acid, and Bial(4) could find no trace of solid urate in these cells. The bird cannot afford to lose even the minimum amount of water in which the daily output of uric acid could be dissolved, and hence some device must be adopted for re-absorption of water. If concentration of uric acid in the renal tubules were not limited, sufficient deposit might be formed there to interfere seriously with excretion. This is prevented by rapid diuresis, and loss of water is avoided by cloacal re-absorption. Occasionally fowls under ether or urethane anæsthesia exhibit complete anuria, and no diuretic will induce the kidneys to resume their function. This may indicate blocking of the renal tubules, resulting from slow passages of urine; the condition, however, has not been investigated.

The relative insolubility of uric acid, therefore, renders necessary continuous diuresis and re-absorption of water in the cloaca. It is not quite so clear whether any advantage would be gained by specific secretion of uric acid; but the possibility of this process in the bird deserves consideration. Sharpe(5) has found that adrenalin causes diuresis in fowls, and this is remarkable in view of its opposite effect in mammals. An increase in uric acid concentration may sometimes accompany the diuresis, and seems occasionally to occur with other diuretics also(6). This, too, is in contrast with mammalian excretion. The rapid elimination of fluid by the kidney, after injections of adrenalin, might be explained by supposing that local vaso-constriction is less complete or of shorter duration than that in other parts; the kidney would then obtain a good supply of blood at a higher pressure than usual. But a coincident increase in the concentration of uric acid seems to involve some secondary action on the renal cells; more probably an effect of the greater filtration than a direct action of the diuretic. Such evidence as is available, therefore, does not indicate much resemblance between the renal processes of birds and mammals.

Among methods employed to examine the action of the kidney in mammals two appear to be of special value, namely, comparison of the increase in concentration experienced by several waste products during their excretion; and observation of the changes produced in the urine when its outflow is resisted. Some experiments have now been carried out in which these tests were applied to the excretory function of birds.

Method. Cockerels were anæsthetised with ether or urethane, and the ureters were exposed by an abdominal incision. Cannulæ were introduced and the urine collected. Sometimes, when the fluid was not quite clear, intravenous injections of adrenalin were given, until the flow was sufficiently rapid to prevent the formation of deposit. This was not invariably done, however, and urines which contained a deposit of urates are marked with an asterisk. Blood was taken from the sciatic artery. Clotting is slow unless contamination with tissue juice has occurred; and as a rule no anti-coagulant was used, but the blood was collected in paraffined tubes, kept on ice, and centrifuged as soon as possible. The presence of oxalate or citrate is undesirable when certain colorimetric estimations are necessary.

The concentrations of uric acid and phosphate in the plasma and urine were compared; in later experiments creatine, creatinine, and chloride were examined in the same way, and occasionally urea and ammonia also. Removal of blood was postponed until sufficient urine had been obtained, in order that interference with excretion should be avoided. The collection of urine had, therefore, to be limited to a period during which the plasma remained fairly constant in composition. This condition appears to be fulfilled for intervals of at least 20 minutes. Thus, a particular sample of plasma contained 4·4 mgm. of uric acid and 3·4 mgm. of phosphate per 100 c.c. and a second sample from blood removed 20 minutes later contained 4·3 mgm. of uric acid and 3·0 mgm. of phosphate. Urine was generally collected for much shorter periods, however, since the amount required was often excreted in 1 to 5 minutes. Similar methods of analysis were employed for plasma and urine; the latter being diluted, when necessary, to about the same concentration as the former. Uric acid was estimated in the manner recently described by Benedict(7); phosphate by Bell and Doisy's method(8); creatine and creatinine by the method of Folin and Wu(9); chloride by Wetmore's application of Volhard's procedure(10); ammonia by the vacuum distillation process of Kruger, Reich and Schittenhelm(11); and urea in a similar manner, after hydrolysis with urease.

In three experiments the flow of urine from one kidney was resisted by using a long ureteral cannula which could be bent upwards to a vertical position. By this means pressures corresponding to about 15–25 mm. of mercury were applied, and the concentrations of uric acid and phosphate found in the urine were compared with those of normal urine excreted simultaneously by the other kidney.

In several of the earlier experiments samples of plasma were ultra-

filtered, in order to test the diffusibility of the uric acid and phosphate which they contained; for if part were attached to colloids the failure to recognise this would invalidate comparisons of their $\frac{\text{urine}}{\text{plasma}}$ ratios, since only the diffusible portions could be excreted. A protein-free fluid was obtained by filtration through small collodion tubes at a pressure of about 10 lbs. per sq. inch[1]. The first portion of the filtrate was rejected in order to avoid dilution. Examples are given in Table I of the concentrations of uric acid and phosphate in several samples of plasma before and after filtration.

TABLE I. *Mgm per* 100 *c c.*

Uric acid		Phosphate (*P*)	
Plasma	Ultra-filtrate	Plasma	Ultra-filtrate
3 4	3 3	2 4	2 1
4 6	4 9	8 1	5 4
6 7	6 8	1·8	1 5
16 1	16 1	5 6	5 4

These results show that the uric acid of fowls' plasma, or that part of it which can be estimated in a tungstic acid filtrate, is able to pass through a collodion membrane. Most of the phosphate can pass through also; a small proportion may be adsorbed on the membrane, for even dilute protein-free phosphate solutions of known strength appear to become weaker during filtration. There is, of course, no proof in either case that part is not attached to colloids removed by precipitation or filtration; but the precipitation methods employed evidently do not interfere with satisfactory measurement of the diffusible portions, and these alone are of importance in connection with excretion[2].

The figures given here and in other parts of this paper indicate that the plasma content both of uric acid and phosphate is subject to considerable variation. Folin and Denis (12) found 4·8 mgm. of uric acid in 100 c.c. of bird's blood, and Robertson (6), using the same method, found 5·15 and 6·16 mgm. Later, Folin and Wu (9) obtained by their more recent procedure amounts varying from 2·5 to 3·8 mgm. per 100 c.c. Most of my own results fall within the somewhat wide range of those reported by other observers, but there is no doubt that, under anæsthesia

[1] I am indebted to Dr Dale for suggesting the use of the centrifuge to remove air-bubbles from tubes filled with collodion; and also for pointing out that when the excess of collodion had been poured off 50 p c alcohol could be introduced, without preliminary drying of the film. Suitable thimbles were made by this method in about 10 minutes

[2] White (*Amer. Journ Physiol.* 65. p 200, 1923) assumes that because the phosphate of ox plasma is diffusible this is also true of dogs, and the discordant results which he obtains when comparing normal phosphate excretion with the excretion of injected phosphate may be due to inclusion, in the former case, of phosphate attached to colloids

at any rate, the quantity of uric acid present may occasionally be much greater. The highest concentrations observed exceeded 16 mgm. per 100 c.c. of plasma.

For the purpose of this investigation, however, the absolute quantities of uric acid and phosphate are not of importance, since only their $\frac{\text{urine}}{\text{plasma}}$ ratios are required. These were generally obtained by direct colorimetric comparison of tungstic acid filtrates from plasma with diluted urine to which the same reagents had been added. The actual amounts present were subsequently determined by means of standard solutions. On one occasion 20 c.c. per kilo of 10 p.c. sodium sulphate were injected intravenously, and the concentration of this substance by the kidney was compared with that of uric acid. The second table shows the results of this experiment, as well as of those dealing with uric acid and phosphate.

TABLE II.

No.	Vol. of urine c.c. per min.	Urio acid in plasma mgm. per 100 c.c.	Uric acid in urine mgm. per 100 c.c.	$\frac{\text{Urine uric acid}}{\text{Plasma uric acid}}$	Phosphate in plasma mgm. P per 100 c.c.	Phosphate in urine mgm. P per 100 c.c.	$\frac{\text{Urine phosphate}}{\text{Plasma phosphate}}$	Concentration of uric acid by kidney Phosphate = 1
1	1·70	4·4	67·4	15·3	3·4	9·0	2·6	5·9
2	·44*	4·3	269·8	62·7	3·2	36·5	11·4	5·5
3	·58	3·4	53·9	15·9	2·4	6·9	2·9	5·5
4	·48*	16·1	327·9	20·4	5·6	21·2	3·8	5·4
					Sulphate in plasma gm. Na_2SO_4 per 100 c.c.	Sulphate in urine gm. Na_2SO_4 per 100 c.c.	$\frac{\text{Urine sulphate}}{\text{Plasma sulphate}}$	Concentration of uric acid by kidney Sulphate = 1
5	·30	6·4	84·6	13·2	·62	1·24	2·0	6·6

It will be seen in Table II that the $\frac{\text{urine}}{\text{plasma}}$ concentration ratio of uric acid is more than five times as high as that of phosphate, and more than six times as high as that of sulphate. If this means that uric acid is secreted, the constancy of these relations suggests that phosphate and sulphate are concentrated mainly by re-absorption of water. The possibility of secretion of phosphate will, however, be considered later. Experiments in which the urine contained a deposit can be criticised on the ground that solid material may accumulate for some time in the tubules and leave the kidney at intervals, thus producing a sudden increase in the uric acid content of the urine and rendering it useless as a measure of the concentrating power of the kidney. If this were so, the relation between the concentration ratios of uric acid and phosphate would not be uniform;

but it is found that this relation remains the same whether deposit is present or not, so long, at least, as the flow of urine is not less rapid than those recorded. In other words, when the urine contains solid uric acid there is an equivalent rise in its phosphate content. Hence, there is reason to believe that no accumulation of uric acid in the tubules has occurred.

The results obtained by applying a similar method of investigation to other constituents of the plasma and urine are grouped together in Table III.

TABLE III.

No.	Vol. of urine c.c. per min.	Plasma concentrations mgm. per 100 c.c.					Urine concentrations mgm. per 100 c.c.					Urine / Plasma				
		Uric acid	Phosphate (*P*)	Creatine	Creatinine	Chloride (NaCl)	Uric acid	Phosphate (*P*)	Creatine	Creatinine	Chloride (NaCl)	Uric acid	Phosphate	Creatine	Creatinine	Chl ri
1	·55	8·6	—	6·4	1·5	—	124·8	—	17·4	4·1	—	14·5	—	2·7	2·7	—
2	·38	6·1	1·2	5·1	1·3	670·0	197·9	Nil	14·1	3·9	240·0	32·4	—	2·8	3·0	·3
3	·68*	—	4·9	4·0	1·3	690·0	—	36·5	22·5	5·4	20·0	—	7·4	5·6	4·2	·0

Urea and ammonia are not included in this table, because the method of estimation employed was not found sufficiently delicate to give very accurate results with the quantities of plasma available. The total urea and ammonia nitrogen of the plasma does not appear to exceed 0·7 mgm. per 100 c.c. In urine collected from the ureters as much as 28 mgm. per 100 c.c. may be present; in one sample four-fifths of the total was in the form of ammonia, but there is no evidence that this is always the case. At any rate urea and ammonia are only excreted in small amount by the bird, and are of relatively little importance. Table III shows that uric acid is the principal waste product in the urine. It is well known that creatine is largely excreted unchanged; the quantitative relation between this substance and creatinine is indicated in the table. Chloride is at a lower concentration in the urine than in the plasma, and the very limited excretion of chloride in Exp. 3 suggests that it may sometimes be absent from concentrated urines. Phosphates also may apparently be retained, for none could be detected in the urine of Exp. 2, when the plasma content was only 1·2 mgm. per 100 c.c.

It is probable that this investigation has included all the important waste products excreted by the kidney of the bird, and the fact of most interest is that uric acid, during elimination, undergoes a much greater increase in concentration than any other substance examined. Urea and ammonia may perhaps be exceptions; the results obtained in connection with excretion of nitrogen in these forms indicate that a

$\frac{\text{urine}}{\text{plasma}}$ ratio of 40 is possible. But in view of the statement by Davy (13) that nearly all the ammonia of birds' urine is in combination with uric acid, this observation is not of much value in deciding the mechanism of excretion; for the substances to be compared must obviously be independent of one another. Creatine and creatinine seem to be slightly inferior to phosphate as regards the efficiency of their elimination. Chloride is not concentrated at all and is, therefore, of no interest for comparison.

Uric acid, then, experiences a greater increase in concentration than any other substance which passes from the plasma into the urine. This can only be explained by admitting secretion of uric acid, or by postulating extensive re-absorption of everything else. The secretion theory is, of course, much more probable, but these experiments furnish no evidence as to whether anything except uric acid is secreted. In any case, uric acid is in a class by itself; in the bird there is no group of substances which conforms to Cushny's definition of no-threshold bodies.

The figures given in Table III are of interest from another aspect. When the concentrations of the plasma and urine are calculated from a physical standpoint, it is clear that the fluid which leaves the kidney of the bird must be hypotonic. The total force exerted by the kidney in overcoming osmotic tension is, therefore, on the whole applied in a direction opposite to that which ordinarily characterises mammalian excretion. Chloride is, of course, the deciding factor, and since this substance may be almost absent from the urine, although it probably passes through Bowman's capsule at plasma concentration, there is good reason to suppose that it is re-absorbed in the renal tubules. Hence it seems to be in re-absorption that the anomaly occurs; the mammal generally increases the osmotic pressure of the tubule fluid by absorption (since relatively little urea is absorbed), but the bird appears to reduce it by the same process. The hypotonicity is of importance in the analogy to other secretions which it suggests.

Uric acid is excreted so efficiently that the amount lost by the plasma in the renal circulation is probably considerable. Water is eliminated also, and it is evident that an observed decrease in uric acid concentration cannot be so great as to represent the true loss of uric acid. However, an attempt was made to obtain blood from the renal veins for comparison with arterial blood. This is difficult owing to the impossibility of ligaturing other veins which communicate with the renal. The coccygeomesenteric vein lies on the anterior surface of the kidney, and is closely attached to this surface throughout almost the entire length of the organ;

the renal blood is carried to this vein by vessels which pierce its posterior wall as soon as they leave the kidney. The femoral vein is also a tributary of the hypogastric. Ultimately the renal circulation was isolated by tying the femoral vein and inserting a cannula into the proximal portion; then enlarging the opening through which this vessel enters the abdomen and applying digital pressure to the hypogastric vein at the upper and lower poles of the kidney, with two fingers introduced through the opening. As soon as the upper point was compressed, renal venous blood was allowed to flow from the cannula, and was collected for a short period. The circulation through the kidney is evidently slower than in mammals About 5 c.c. of venous blood were obtained in 2 or 3 minutes. Arterial blood taken from the sciatic artery in the usual way is the same as that which supplies the kidney. The arterial sample of plasma collected was found to contain 13·2 mgm. of uric acid per 100 c.c. and the venous sample 10·3 mgm. Hence, there was a loss of 3 mgm. per 100 c c without allowing for concentration of the blood by removal of water; or nearly a quarter of the uric acid brought to the kidney. When the concentration factor is considered the efficiency of excretion is still more evident.

The remaining experiments deal with the effect of resisting the outflow of urine; the method employed was quite simple, and has already been described. At first the pressure was applied to one ureter only, and the urine compared with that excreted normally by the other kidney But with no interference at all the minute volumes and concentrations of the urines from the two sides may be quite different, and the urine from either kidney is subject to rapid variations in character. In a later experiment, therefore, each kidney in turn was made to excrete against resistance, and comparison was thus possible with the fluid excreted before and after the period of pressure, as well as with the normal output of the unresisted kidney during this period. The results are given in Table IV.

TABLE IV.

	Vol of urine c c per min		Uric acid mgm per 100 c c		Phosphate mgm *P* per 100 c c	
No	Normal	Pressure	Normal	Pressure	Normal	Pressure
1	·48*	·08	327 9	269 7	21 2	13 2
2 (*a*)	62 ·70	—	106 7 82 0	—	14 8 14 6	—
(*b*)	·18*	·03*	218 4	229 9	28 4	35 3
(*c*)	·19*	—	565 6	—	15 7	—
3 (*a*)	53* ·15	—	253 2 178 9	—	17 0 10 6	—
(*b*)	·40	·10*	207 5	361 4	9 8	32 3
(*c*)	51	—	346 8	—	18 8	—
(*d*)	58	08	175 5	232 6	11 5	11 3
(*e*)	1 17	—	195 7	—	6 9	—

and this may indicate that different groups of cells are concerned in their secretion.

(6) The urine is hypotonic, and in this respect resembles the product of a secreting gland.

These considerations leave little doubt that the kidney of the bird can secrete. Re-absorption is possible also, although the use of the cloaca for this purpose renders the process less necessary than in the mammalian kidney. The effect of resistance in causing increased concentration of the urine seems to indicate that water can be absorbed from the renal tubules. Chloride and occasionally phosphate may be re-absorbed, for the urine may contain less than the plasma. In Exp. 5, Table II, there was 1·24 p.c. of sulphate in the urine. If any re-absorption of water occurred in this case (and it probably always occurs to some extent), the last stage of concentration was almost certainly attained by this process. Hence, the tubule cells can remove water against great osmotic *resistance when they are called upon to do so.*

Since birds and mammals have a common ancestry, it seems reasonable, at first sight, to assume that if the kidney of the bird can secrete so also can the mammalian kidney; and to ignore the absence of satisfactory proof of secretion in the mammal. But there are serious objections to the analogy. None of the evidence of secretion obtained in this investigation is applicable to mammals, in which nearly all the observations described have already been made with opposite results. In the mammal several waste products (no-threshold bodies) experience an almost equal increase in concentration while they are being excreted, and hence are probably concentrated by re-absorption of water. The urine from the two kidneys is generally similar in amount, and when some difference does occur in the rate of excretion the concentration of no-threshold bodies is either the same on both sides or somewhat greater in the smaller volume. Evidently the rate at which glomerular filtrate flows through individual tubules is usually about the same in both kidneys, and the quantity of urine is largely determined by the number of excreting units in action. Roughly the same proportion of fluid is therefore re-absorbed in both cases. If secretion were possible, local variations in cell activity would probably occur, and produce results similar to those described in birds. Diuresis reduces the concentration of the urine in mammals, and a slow outflow causes increased concentration. When one kidney is excreting against pressure its urine is more concentrated than that of the other, as regards no-threshold constituents at any rate[1]. This is to

[1] The discrepancies in the results obtained by different observers as regards the effects

be expected in the absence of secretion, since more time is allowed for the re-absorption of water The urine of mammals is nearly always hypertonic, for even when a considerable quantity of chloride is being retained the concentration of urea and no threshold bodies ensures hypertonicity In this characteristic the urine differs from most secretions

In conclusion, then, it is clear that the bird has developed a distinctive excretory mechanism, in which selective re absorption by the kidney plays only a minor part No great quantity is found in the urine of any substance which can offer osmotic resistance to concentration A relatively insoluble waste product is excreted in large amount, and the process involved would not necessarily be suitable for the excretion of soluble substances at a high concentration, such as the kidney of mammals can produce

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of resistance on the volume of urine appear to be explained by the experiments of Lucas *Amer Journ Physiol* 22 p 245, 1908, who found that in the dog moderate resistances do not always increase the pressure in the renal pelvis In such cases the volume of urine may be greater owing to a nerve reflex from the ureter, but it is probable that when the pressure is actually raised in the pelvis, the kidney, if normal, always excretes less urine and the concentration of no threshold bodies is necessarily increased

SERIES A. Pressures in kilos. The successive numbers give the results of successive *stimulations.*

	With circulation		Without circulation	
Exp. 1. Median nerve	Skin and deep tissues	Deep tissues only (skin frozen)	Skin and deep tissues	Deep tissues only (skin frozen)
Normal ...	8, 8, 8, 8	10, 10, 10	10, 10, 10	10, 10, 10
Weak stim. ...	6, 6, $5\frac{1}{4}$, $6\frac{3}{4}$	5, 5, 5	$6\frac{1}{4}$, $6\frac{1}{4}$, $6\frac{3}{4}$, $6\frac{3}{4}$	7, 8, 8
Moderate stim.	7, 6, 6	7, 7, 7,	6, 6	10, 10, 9
Strong stim. ...	$8\frac{3}{4}$, 10, $9\frac{1}{2}$, $8\frac{3}{4}$, 10, 10	9, 9, 10	9, 11, 9, 10	12, 13, 12
Exp. 2.				
Normal ...	8, 9, 10, 9, 8	10, 10, 10	7, 8, 7	9, 9, 9
Weak stim. ...	$6\frac{1}{2}$, 7, 6, $7\frac{1}{2}$	$6\frac{1}{4}$, $6\frac{1}{2}$, $6\frac{1}{4}$, 7	$4\frac{3}{4}$, $5\frac{3}{4}$, $4\frac{1}{2}$, $4\frac{3}{4}$	$6\frac{1}{4}$, 6, $6\frac{1}{2}$
Moderate stim.	4, 5, 4, 6, 5	9, 9, 9	$6\frac{3}{4}$, $6\frac{3}{4}$, $6\frac{3}{4}$, $6\frac{3}{4}$	10, 14, 11, 12
Strong stim. ...	—	—	—	—

Very strong stimulation. Very strong nerve stimulation following a weak stimulation, increases the threshold for pressure pain and in some cases raises it above that present before the weak stimulus was applied. Thus in Exp. 1 pain was felt normally with a pressure of 8 kgm., weak stimulation lowered it so that 6 kgm. was sufficient to produce pain, whilst immediately after very strong nerve stimulation 9·3 kgm. was required. Similar results were obtained when the skin was anæsthetic and deep sensibility only left. The stimulation causes also very marked blanching of the skin and, as with the weak stimulation, the question arises whether the changed sensibility is caused by the changed blood flow. In order to decide this the same method as with the weak stimulation was employed. The circulation was stopped by an arm bandage and during the cessation of the circulation the median nerve was stimulated with strong induction shocks, it caused a marked increase in the threshold for pressure pain. I conclude therefore that the decreased sensibility is not in the main due to vaso-constriction.

Moderately strong nerve stimulation (*i.e.* currents, which whilst causing vigorous contraction, were comfortably borne). Faradic stimulation of this degree produced marked pallor and lowered the threshold, but it differed in its effects from weak stimulation:

(*a*) In causing in most cases a greater decrease of the threshold for pressure pain. This was especially as in Exp. 2. With weak stimulation a pressure of 6·5 to 7 kgm. was required whereas with moderately strong stimulation the pressure required was only 4 to 6 kgm.

(*b*) In causing during cutaneous anæsthesia a less decrease of the threshold than is caused by weak stimulation. Thus in Exp. 3 during cutaneous anæsthesia weak stimulation reduced the pressure required

from 10 to $6\frac{1}{2}$ kgm., whilst moderately strong stimulation reduced it to 9 kgm. only.

It appears then that moderately strong stimulation increases skin sensibility much more than it increases deep sensibility.

Since vascular changes do not account for the changes in pressure pain sensibility caused by nerve stimulation some other explanation must be sought for. The explanation might conceivably lie in a change of conductivity of the nerve fibres in the stimulated region; whilst it is possible that some decrease of conductivity is caused by strong currents there does not seem to be any evidence that it can be increased by weak currents and it will be seen from what follows that there is evidence that the chief cause of the change in the threshold of pressure pain is a change in the central nervous system.

The nerve stimulation necessarily sets up impulses which affect the central nervous system. Since afferent impulses set up on one side of the body readily affect the nerve centres of the opposite side, either exciting or inhibiting them, the state of the palm of the hand was noted on the opposite side to that on which the median nerve was stimulated. It was found that there were in general changes in the pressure pain sensibility on the opposite palm of the same nature as those produced on the stimulated side. With weak stimulation there was increased sensibility on the opposite side in five out of six cases, and on several occasions flushing was observed in the thenar region on the opposite hand to that stimulated, although this was never very marked. With strong stimulation on one side, the sensibility on the opposite side diminished in five cases.

SERIES B. Pressure in kilos. The successive numbers give the results of successive stimulations.

Stimulus	Right thenar	Right hypo-thenar	Left thenar	Left hypo-thenar
Exp. 1.				
Normal ...	8, $8\frac{1}{2}$, 8, 8	9, $9\frac{1}{2}$	8, $8\frac{1}{2}$, 8	$8\frac{1}{2}$
Weak stim. ...	6	$5\frac{3}{4}$	$4\frac{3}{4}$, $5\frac{3}{4}$, $5\frac{1}{4}$	$6\frac{1}{4}$, $7\frac{1}{4}$
Moderate stim.	7	7	$7\frac{1}{2}$	8
Strong stim....	10, 9	10, 9, 9	10, 9, 8	8, 8, 9
Exp. 2.				
Normal ...	7, 7, $6\frac{3}{4}$	8, $7\frac{1}{2}$, 7	8, 8, $7\frac{3}{4}$	8, 8, 7, $7\frac{3}{4}$
Weak stim. ...	5, 5, 5	6, $5\frac{1}{2}$, $5\frac{1}{2}$, 5	6, $5\frac{1}{2}$, 5, 5	6, $5\frac{1}{2}$, $5\frac{1}{2}$
Moderate stim.	6, 6, $5\frac{3}{4}$	6, $6\frac{1}{2}$, 6	6, 6, 6	$6\frac{1}{4}$, $6\frac{1}{2}$
Strong stim....	$8\frac{3}{4}$, 8	$8\frac{1}{2}$, 8, 8	$7\frac{1}{2}$, 7	8, $7\frac{3}{4}$, 7

In some instances the strong stimulation caused pallor in both arms and widespread sweating; and in one subject there was the interesting result that the sweating both on the stimulated and on the opposite side

INTERRELATION OF PARATHYROIDS, SUPRARENALS AND PANCREAS. By G. A. CLARK.

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THAT the parathyroid glands have an influence on carbohydrate metabolism has been indicated by the experimental results of numerous workers, notably by Underhill and his associates (1), who showed that removal of the parathyroids brought about a hypoglycæmia with disappearance of liver glycogen. They consider that these conditions are neither the cause nor the effect of the tetany resulting from parathyroidectomy. It is well known that the guanidine content of the blood is increased after parathyroidectomy and also that administration of guanidine to the normal animal can produce symptoms similar to those of tetany (2), including profound changes in the blood-sugar. These blood-sugar changes have been investigated by Watanabe (3) who, using doses of guanidine sufficient to cause symptoms of tetany, showed that a single injection produced a hyperglycæmia, followed in about 7 hours by a hypoglycæmia. A second dose several days after the first resulted in a hyperglycæmia without any subsequent lowering of blood-sugar, unless a lethal dose were administered, in which case hypoglycæmia was observed just before death. Glycosuria was not found. That guanidine may have a direct action on blood-sugar is suggested by the fact that guanidine salts added to dilute glucose solutions alter the ratio of the polarimeter value to the copper-reducing value as determined by Bang's method (4).

The present investigation was begun to determine the effect of guanidine on the blood-sugar of rabbits, avoiding as far as possible the production of gross symptoms of tetany. The dose was therefore limited to ·1 gm. per kilo. of body-weight. It should be noted, however, that Paton and Findlay have in some cases found this amount sufficient to cause an increase in electrical excitability in rabbits (2). Guanidine hydrochloride was injected in concentrated aqueous solution into the marginal vein of one ear, while blood for sugar estimation was obtained by vene ection from the opposite ear every hour or half-hour. The majority of animals showed no abnormal signs after an injection but

in a few cases shaking of the forepaws, movements indicating irritation of the mouth or noso and increased salivation were ohserved; on two occasions dyspnœa was present. In all cases the rahbits appeared normal at the end of an hour. None of the animals had food for 18 hours before an experiment. For the estimation of blood-sugar Bang's old micro-method was used, a small platinum capsule, as in Calvert's method (5), heing utilised in which to collect and weigh the blood in place of the nsual absorhent paper. This procedure shortened considerably the time required for cach estimation. Before every experiment two and often three samples of blood werc taken, at intervals of half-an-hour, to determine the normal sugar content. Throughout the paper, unless otherwise stated, sugar values are givcn in mgms. per 100 mgms. of blood. In Fig. 1 is given one typical result from each of the first four series of experiments; a summary of other results is given at the end of the paper.

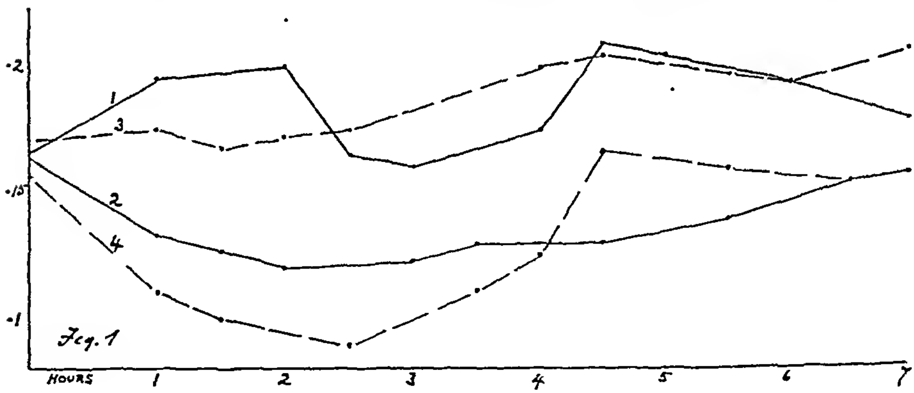
Effect of a single dose of guanidinc hydrochloride. A first injection of guanidine causes the blood-sugar to vary in an apparently erratic mannor, hut the curves depicting these variations show a ccrtain similarity in that a rise occurs during the first hour, followed hy a fall of varying duration. In two cases this fall is succceded by a second rise; in all there is a tendency to revert to the original levcl after 6 or 7 hours (Curvc 1, Fig. 1). That conflicting factors were at work to produce these results is ohvious and, as it is known that guanidine has a stimulating action on the sympathetic nervous system (6), it was reasonable to suppose that the hyperglycæmia was of the adrenaline type. Miculicich (7) and Burn (8) showed that adrenaline hyperglycæmia is inhibited by ergotoxinc. In a second series of experiments, therefore, each rabbit was given a preliminary dose of 3 mgms. ergotamine tartrate, which has been shown by Dale and Spiro to have the same pharmacological action as ergotoxine (9). Ono hour later guanidine was injected and blood-sugar estimations carried out as before. An immediate fall in blood-sugar occurred and persisted in two animals for at least 7 hours (Curve 2, Fig. 1). In the third case the normal level was regained after 3½ hours.

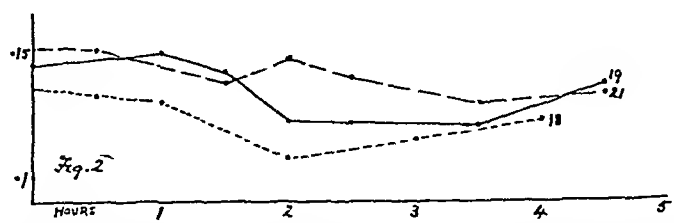
Effect of a second dose. Two rabbits received a second injection of guanidine alone, 10 weeks and 12 weeks respectively after the first. In both cases a hyperglycæmia resulted without any fall of the blood-sugar below normal and in one animal the original level was not regained for 25 hours (Curve 3, Fig. 1). That this hyperglycæmia is due to hyperactivity of the suprarenals or to a lowered threshold to stimuli of the sympathetic system, brought about by the first dose, is suggested by the following experiments: Three rabbits received a dose of ergotamine

tartrate an hour before a second injection of guanidine, which now produced an immediate fall in blood-sugar, more abrupt than that seen after a first dose, but the normal level was regained in all cases after 4½ hours (Curve 4, Fig. 1). Ergotamine alone was not found to have any marked influence on blood-sugar.

That this return to normal was not due to waning of the ergotamine action on the sympathetic was shown by the fact that in one rabbit a second dose of ergotamine was given when the blood-sugar was at a minimum; no prolongation of hypoglycæmia followed. Thus, if the normal supply of glucose from the liver is unavailable, guanidine produces an immediate fall in blood-sugar. This is further illustrated by two experiments in which guanidine was administered after the supply of glycogen had been depleted by starvation and the injection of strychnine on the day previous to that of the experiment. As will be seen from the following results, the hypoglycæmia is less than that after ergotamine, probably because of the difficulty of ensuring complete exhaustion of the glycogen supply:

Hours after injection	0	1	2	3	4	5	6
No. 3	·150	·141	·133	·141	·140	·138	·139
No. 15	·155	·122	·116	·125	·135	·133	·130





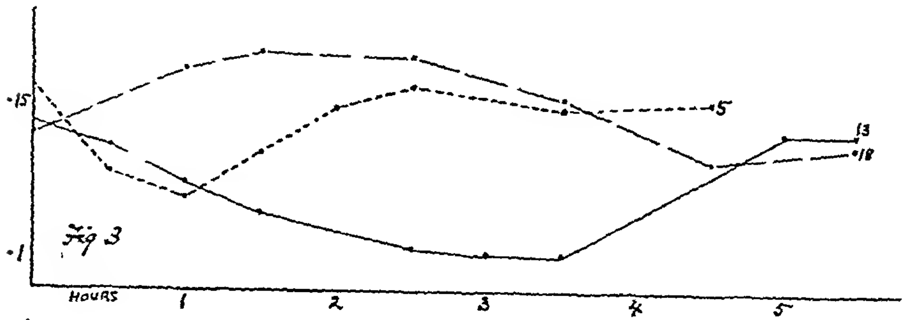
On the other hand, as long as glycogen is available, guanidine will cause its liberation from the liver by sympathetic stimulation. It appears

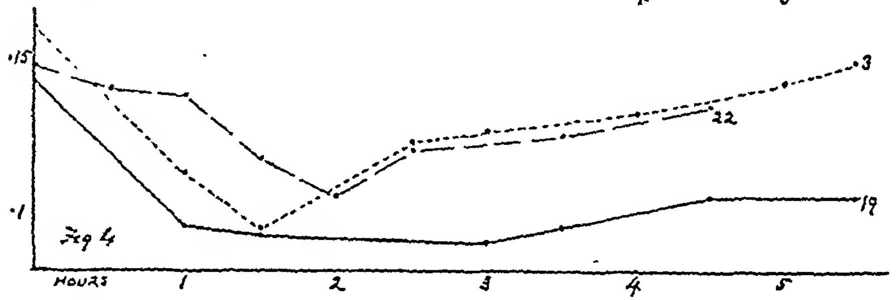
from Watanabe's work (3) and from the above results that this glycogenolytic action is stronger than the hypoglycæmic action, so that, if guanidine is the cause of the low blood-sugar observed after parathyroidectomy, it can only be so when the liver no longer contains glycogen.

Burns and Watson produced evidence to show that guanidine salts have a nicotine-like action on the vagus, first stimulating and later paralysing the fibres to the heart (10). It is therefore reasonable to suppose that other branches of the vagus are similarly affected. McCormick and O'Brien showed that it is possible to produce a lowering of blood-sugar in animals by stimulation of the right vagus, taking precautions to avoid cardio-inhibitory effects (11). The nerve supply to the islets of Langerhans is believed to come in large part from this nerve and the hypoglycæmia resulting from its stimulation can be ascribed to liberation of insulin. It is possible to explain the hypoglycæmic action of guanidine in this way, and three experiments were carried out to determine this point. The rabbits were given 3 mgms. of ergotamine tartrate one hour before, and ·15 mgm. of atropine sulphate intravenously 15 minutes before an injection of guanidine hydrochloride; ·05 mgm. of atropine was also given at the same time as the guanidine. From Fig. 2 it is seen that No. 19 showed no hypoglycæmia for 1½ hours, and that the fall in blood-sugar occurring then is considerably less than that observed in parallel experiments without atropine (Curve 4, Fig. 1). A similar result was obtained in No. 18. No. 21 was given a further injection of atropine sulphate (·15 mgm.) 1½ hours after the guanidine, producing a slight rise in blood-sugar half-an-hour later, which, however, does not reach above the normal level.

Effect of calcium on guanidine hypoglycæmia. The administration of calcium relieves the symptoms of tetany after parathyroidectomy and Underhill and Blatherwick found that in dogs the accompanying hypoglycæmia was also diminished (12). Watanabe on the other hand was unable to restore the blood-sugar to normal by injections of calcium lactate during guanidine hypoglycæmia (13). In the present investigation calcium chloride was given intravenously, the animals having had ergotamine. Rabbit 13 received 1 c.c. of a 1·5 p.c. solution at the same time as the guanidine; the resulting fall in blood-sugar (Fig. 3) is neither so abrupt nor so great as that seen without calcium (Curve 4, Fig. 1). Rabbit 18 was given 2 c.c. with guanidine and the result is in this case a transient hyperglycæmia. In a third animal a similar dose of calcium was given during the guanidine hypoglycæmia, with the result that the

blood-sugar regained its normal level within 1½ hours. Calcium alone after ergotamine was not found to have any effect on blood-sugar.





Effect of parathyroid extract. The preparation used was the Parathyroid Tablets 1/10 gr. (Armour). These were triturated with normal saline and the fluid obtained after filtering or lightly centrifuging was given intravenously. Three rabbits were employed, ergotamine having been injected one hour before guanidine. No. 19 was given the extract from three tablets at the same time as a first dose of guanidine. The resulting hypoglycæmia appears to be accentuated rather than relieved (Fig. 4). In No. 3 the extract of four tablets with guanidine caused a more rapid fall in blood-sugar and a more rapid initial recovery, but complete recovery was somewhat delayed. The remaining rabbit was given two parathyroid tablets by mouth on the morning and evening of the day before the experiment, and also an intravenous injection of the extract of three tablets with the guanidine. In this case the fall in blood-sugar was delayed, and the recovery was similar in type to that of No. 3. The parathyroid extract employed was found to have no influence on blood-sugar when given alone after ergotamine.

Discussion. From the foregoing experiments it is seen that guanidine produces two effects, the first and more powerful being a stimulation of the sympathetic system causing a hyperglycæmia, which is probably augmented by an increased production of adrenaline, and the second a

hypoglycæmia which can be explained by an increased liberation of insulin due to vagal stimulation. The action of a single injection of guanidine seems to render the sympathetic nerves more susceptible to further doses of guanidine. A similar sensitising action on the vagus is suggested by a comparison of Curves 2 and 4, Fig. 1. The fall in blood-sugar after a second injection of guanidine following ergotamine is greater and more rapid than that after a first injection and the equally rapid recovery in the former case may be due either to the supervention of vagal paralysis or to the fact that the whole of the available insulin has been discharged from the islets. That guanidine causes an increase in the ratio of polarimeter value to copper value when added to dilute glucose solutions *in vitro* has already been described (4). If it is possible to cause exhaustion of the islets by guanidine, it is evident that, because of the hyperglycæmia simultaneously produced by stimulation of the sympathetic system, a blood condition similar to that of diabetes will occur. This possibility is being investigated and the following experiment is suggestive: A male rabbit was given five doses of guanidine over a period of 10 days. The animal's weight fell from 2·4 to 2·1 kilos. Three days after the last injection the blood-sugar was found to be ·183, the value at the beginning of the experiment being ·138. The rabbit was killed and the blood-sugar extracted according to the method used by Winter and Smith (14). The initial polarimeter value of the extract was equivalent to ·104 p.c. but fell to coincide with the Bang value at ·056 p.c. after 38 hours. No sugar was detected in the urine.

It is known that the sugar in diabetic blood is present in a form which cannot be readily utilised by the organism; the blood-sugar in adrenaline hyperglycæmia shows a similar ratio of polarimeter value to copper value (15). It may be inferred then that glucose as liberated from the liver must be altered in some way by insulin to render it readily assimilable by the tissues, so that an increased glycogenolysis will need an increased production of insulin. It is of value therefore that the same factor which liberates glucose from the liver should also control the supply of insulin.

The apparently contradictory results obtained with parathyroid extract may be explained in the light of recent experiments by Winter and Smith, showing that parathyroid extract augments the action of insulin (16). The effect of parathyroid extract on the blood-sugar in guanidine hypoglycæmia will be the resultant of its action on insulin and that on guanidine.

Summary.

1. Further evidence of sympathetic and parasympathetic stimulation by guanidine is given.

2. Guanidine appears to exert an action on the organism lasting at least 10 to 12 weeks, which alters the balance between glycogenolysis and the production of insulin.

3. The antagonism between calcium and guanidine is illustrated.

4. The results of parathyroid administration appear to show that, when given with guanidine, the extract augments the hypoglycæmia, but, given some time before, delays its onset.

5. A possible factor in the etiology of diabetes is suggested.

I wish to express my thanks to Professor Burns for much valuable criticism and assistance.

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RESULTS OF EXPERIMENTS NOT GIVEN IN FIG. 1.

First dose of guanidine.

Alone			After ergotamine		
Hours after injection	No. 3	No. 4	Hours after injection	No. 10	No. 12
0	·110	·125	0	·140	·130
1	·136	·184	1	·117	·090
1½	—	·151	2	·100	·095
2	·122	·143	3	·084	·105
3	·090	·240	4	·084	·125
4	·100	·215	5	·087	·120
5	·103	·185	6	·095	·120
6	·117	·160	7	·105	—
7	—	·157			

Second dose of guanidine.

	No. 6		No. 5	No. 13
0	·155	0	·158	·160
1	·175	1	·116	·095
2	·160	2	·093	·100
3	·170	3	·120	·105
4	·176	4	·140	·130
5	·188	5	·175	·155
6	·195	6	·150	·165
7	·180	7	·155	—

Out of 24 rabbits only one was found in which it was impossible to produce hypoglycæmia by injection of guanidine after ergotamine or after exhausting the glycogen supply by starvation and strychnine.

THE METABOLISM OF THE SALIVARY GLANDS.
V. The Process of Reconstruction of the Submaxillary Gland.

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PAVLOV, Yandell Henderson and Verchovsky have shown that in experiments on anæsthetised animals, with prolonged stimulation of the chorda tympani, the submaxillary gland excretes more nitrogen than it loses and that this excess of nitrogen is the greater the larger the secretion. The conclusion drawn was that the balance was due to reconstruction of the gland. One of us repeated these experiments and found that the excess nitrogen can be fully accounted for by the non-protein nitrogen of the saliva and that the loss of nitrogen or reducing substance by the gland is always equal to the protein nitrogen and the reducing substance of the saliva (1,2). Thus no formation of protein cell substance could be detected. Most of what is known about the rate of reconstruction of an exhausted gland has been obtained by histological means. This communication reports some experiments on reconstruction performed with physiological methods.

Method. The dog which was used for these experiments weighed 20 kilos. at the beginning and 21·5 kilos. at the end of the experiments in sixteen months. A fistula of the left submaxillary duct and a gastric fistula were established three weeks before the commencement of the experiments. In each experiment, after a prolonged rest of the digestive organs the animal was placed in a stand, fed with small pieces of dried bread, and the saliva collected in measuring cylinders. The feeding continued for many hours, but in no case was the animal forced to take the food. At different intervals of time (generally once every hour), the gastric fistula was opened and all the gastric contents removed. Distension of the stomach was thus prevented and the animal continued to eat for a very long time. The experiment generally had to be terminated on account of muscular fatigue. Water to drink was given before and after the experiment; during the experiment water was injected through the gastric fistula directly into the stomach.

The first 10 c.c. of saliva were always rejected in order to avoid any possible admixture of stagnant saliva. The subsequent secretion was

usually collected in three or four portions; the first, about 30 c.c. at the beginning, then a large second portion, and a third, also about 30 c.c., at the end of the experiment: on some occasions this was followed by a similar fourth portion. The determinations made were: (1) total nitrogen, (2) non-protein nitrogen, (3) reducing substance in acid hydrolysates of the saliva, (4) and in some cases the protein nitrogen, in other cases the amount was calculated. The non-protein nitrogen was determined in filtrates of the saliva after precipitation of the proteins with alcohol. The reducing substance was determined as described by one of us in a previous communication (2). That partial exhaustion of the submaxillary gland can be obtained by prolonged feeding was first demonstrated by Volborth (3), and our interest was mainly directed to the phase of the reconstruction of the exhausted gland. It was postulated that the composition of saliva which is secreted in response to the same stimulus is a fair indication of the degree of the recovery of the gland. To determine the rate of the recovery 20 c.c. samples of saliva were collected at different times after the termination of the period of exhaustion. To obtain the saliva the dog was again fed for short periods with dried bread similar to that which was used for the exhaustion. 10 c.c. of saliva were in this case also rejected before the collection of the actual sample. The composition of these samples was compared with that of the first sample which was obtained at the beginning of the exhausting period and the gland was considered to have fully recovered when the samples reached the composition of the saliva before the exhaustion. Except for the small amount of dry bread which was given to collect the samples of saliva, the animal received food during the period of recovery through the gastric fistula only.

The rate of recovery of the glandular tissue has never yet been determined, and as this was expected to be rapid, the collection of the post-exhaustion samples was at first made at intervals of two hours. Only 5 c.c. samples were collected, as it was thought that a collection of large samples at such short intervals might impede the process of recovery. It was soon found, however, that the reconstruction of the gland is a very slow process, so that the intervals between the samples could be prolonged to twelve and more hours: 30 c.c. of saliva (20 c.c. sample and 10 c.c. rejected) repeatedly secreted at such long intervals do not cause any exhaustion of the gland by themselves (Exp. 1).

Exp. 1. Four samples of saliva of 35 c.c. collected at intervals of 12 hours.

Rate of secretion	0·8	1·12	0 9	1·0
Total N_2 mgm. p.c.	74	70	72	74

Exp. 1, which was repeated several times, shows no trace of exhaustion of the gland when the saliva is secreted at sufficiently long intervals. It shows, moreover, that small variations in the rate of secretion do not materially influence the composition of the saliva. In experiments with feeding it was found to be much more difficult to maintain a uniform rate of secretion than in experiments with direct stimulation of the chorda tympani. Fortunately, however, even greater variations in the rate of the secretion, provided that samples were not very small, did not affect the composition of the saliva to such an extent as to introduce an appreciable error in the experiments. Exp. 2 shows that the Heidenhain phenomenon is not marked under the conditions of the experiments. However, in none of the experiments did the rate of secretion fall to a half.

Exp. 2. The four samples of saliva were collected at intervals of 10–12 hours.

c.c. of saliva collected	Rate of secretion	Total N_2 mgm. p.c.
31	0·39	70
38	0·67	74
40	0·93	74
40	1·2	78

The exhaustion and recovery of the normal gland. The composition of the submaxillary saliva varies with the different food substances; much care was therefore taken to ensure the same composition of the dry bread and especially with regard to its water content. Nevertheless the composition of the saliva which was secreted apparently under similar conditions did show some variations. In 63 determinations the protein nitrogen varied between 50 and 77 mgms. p.c. with a mean for all the determinations of 62 mgms. p.c.; the non-protein nitrogen—between 10 and 16 with a mean of 11·5; the reducing substance—between 120–180 with a mean of 142 mgms. p.c. The lower figures for protein nitrogen and for reduction were invariably obtained when the digestive tract of the dog was not kept at rest for a sufficiently long period.

In sixteen experiments the period of exhaustion of the gland by prolonged feeding ranged from $5\frac{1}{2}$ hours in the shortest experiment to 8 hours in the longest. The total amount of saliva secreted varied with the length of the experiment: the smallest amount collected was 200 c.c. and the largest 375 c.c. The rate of the secretion in every experiment always slowed down towards the end, but in no case was it found to drop to a half. The following are a few typical examples.

Total amount secreted ...	224	287	293	298	302
			c.c. per minute		
Rate of secretion of first sample	1·0	1·03	0·97	1·0	1·1
Rate of secretion of last sample	0·69	0·8	0·68	0·72	0·65

As the secretion proceeds, the saliva becomes poorer in protein nitrogen. The following tables give the results of nine experiments and of two experiments in greater detail.

Exp	Saliva secreted	Mean rate per min	Protein N_2 mgm p c First sample	Protein N_2 mgm p c Last sample	Total protein N_2 secreted	p c of exhaustion
3	247	0 75	53	23	63	56 7
4	226	0 50	51	21	73	58 8
5	296	0 69	57	23	65	60 7
6	232	0 69	67	21	73	68 6
7	217	0 61	50	16	69	69 0
8	298	0 89	73	22	88	69 5
9	287	0 79	70	21	91	70 0
10	293	0 74	68	11	97	83 8
11	320	0 82	77	12	103	84 5

	Exp 4			*Exp* 12			
Sample	1	2	3	1	2	3	4
Saliva c c.	30	166	30	50	50	50	50
Protein N_2 mgm p c	51	31	21	53	50	44	28
[illegible]	11	14	17	10	11	14	17
[illegible]	124	65	44	117	106	66	42
[illegible]	2 0	1 4	1 1	1 85	1 75	1 1	0 9
Ratio reduction : protein N_2	2 4	2 1	2 1	2 2	2 1	1 5	1 5

The non-protein nitrogen was found in most of the experiments to increase slightly towards the end of the secretion This increase was not due to concentration of the blood; this was avoided by administration of water to the animal, as was shown by estimation of the hæmoglobin concentration in the bloods taken from the jugular vein before and after the experiment. It is probably better explained by an increase in permeability of the glandular cells. One of us determined the non-protein nitrogen in the saliva secreted under the influence of a direct stimulation of the chorda tympani and found that the output of non-protein nitrogen remained constant throughout the secretion The total amount of saliva secreted was generally not much above 100 c.c , but in one experiment with a secretion of 200 c c there was also a rise in the concentration of the non-protein nitrogen.

The concentration of the reducing substance falls as the secretion proceeds. The ratio reduction : total nitrogen drops on account of a relatively larger admixture of the non-protein nitrogen. The small drop in the ratio reduction : protein nitrogen suggests that the globulins present in the submaxillary saliva are exhausted at a relatively slower rate than the mucin

Recovery. The samples of saliva collected at 12-hourly intervals after the end of the period of exhaustion were compared with the first sample

obtained at the beginning of the experiment. The recovery was considered complete when the saliva reached the composition of the latter (Exp. 13).

Exp. 13.

Time of collection	Saliva c.c.	Protein N_2	Non-protein N_2	Total N_2	Reducing substance	*D/N* of saliva	*D/N* of proteins
Exhaustion							
32 mins.	30	70	10	80	170·5	2·1	2·4
5 hrs. 42 mins.	221	38	11	49	80·0	1·6	2·1
41 mins.	30	11	14	25	—	—	—
Recovery. Samples collected at intervals of 12 hours							
32 mins.	30	26	15	41	62·5	1·5	2·4
29 ,,	30	35	10	45	80·0	1·8	2·2
30 ,,	30	46	11	57	—	—	—
35 ,,	30	55	10	65	—	—	—
35 ,,	30	60	10	70	145·0	2·1	2·4
36 ,,	30	65	11	76	153·0	2·0	2·35
34 ,,	30	71	11	81	173·8	2·1	2·45

The experiments show that the recovery of the gland after a large secretion is slow and that the gland does not reach its normal state before the end of the third day. The less the exhaustion the greater is the completion of the recovery as illustrated by the following table.

			p.c. of exhaustion					
Exp.	Amount of saliva secreted	Total N_2 secreted	At end of secretion	12	24	36	48	60 hrs.
14	224	51	36·7	24	10	2	0	—
4	226	73	58·8	50	—	18	12	0
7	217	69	69	50	40	28	—	6
10	293	97	83·8	68·8	49·7	32·3	29	15

Recovery of an atropinised gland. Section of the chorda tympani leads to atrophy of the submaxillary gland accompanied by paralytic secretion. If the secretory fibres of the chorda are those which are necessary for maintenance of the gland in a normal state, then it might be thought that they are also necessary for regulating the process of reconstruction of the gland. We are unable to state whether a disuse atrophy of the submaxillary gland can be produced by a prolonged paralysis of the secretory nerves by atropine; but the process of reconstruction is certainly not retarded by injections of atropine. The experiments were performed in the following manner. After prolonged exhaustion of the submaxillary gland the animal received an injection of a large dose of atropine: the injections were repeated at intervals of a few hours for that length of time found in the preceding experiments to be necessary to ensure a complete recovery of the gland. During the time that the

animal was kept under atropine frequent tests were made to determine whether the gland was completely paralysed. The tests were performed either by giving the animal a small piece of meat or by injecting a little 0·25 p.c. HCl into the mouth. Four separate attempts were successful in producing prolonged and complete paralysis of the gland. Twelve hours after the last injection of atropine a sample of saliva was collected and compared with the composition of the saliva before the exhaustion. Preliminary experiments showed that atropine by itself does not affect the composition of the saliva which is secreted twelve hours later. The four successful experiments with atropine are summarised in the following table:

Exp.	Saliva in c.c.	Protein N_2 before exhaustion	Protein N_2 after exhaustion	p.c. of exhaustion	Hours under atropine	Atropine used mgms.	Protein N_2 in sample 12 hrs. after last injection
15	302	70	15	78	62	182	68
16	287	64	25	61	60	320	61
17	298	68	22	68	63	560	69
18	232	67	21	69	63	760	65

These experiments were performed at intervals of ten to fourteen days. Nevertheless the increased tolerance of the secretory nerves to the drug was well marked, since four times as much atropine had to be used to paralyse the gland in the fourth experiment. The experiments are definite in showing that the recovery is not impeded by paralysis of the secretory fibres of the chorda tympani. In 60 hours the gland was as completely recovered as in the experiments without atropine. The recovery of the reducing substance and the concentration of the non-protein nitrogen does not differ from those in the experiments without atropine.

Exhaustion and recovery after extirpation of the superior cervical ganglion. The superior cervical ganglion was extirpated together with a piece of the vago-sympathetic nerve about 4 cms. long. The experiments were resumed five weeks after the operation and continued for five months. Throughout this period the effects of the extirpation on the pupil and eye were well marked. Malloizel(4,5) and Babkin(6) who studied the salivary secretion after extirpation of the superior cervical ganglion, found a small increase in the mucin content and solids of the saliva. In our experiments there was no change in the content of proteins or of mucin as judged by the reducing substance. However, our experiments differed from the above-named authors' in that they examined the saliva very shortly after the extirpation, while we did it several weeks after. The only definite change observed was in the

concentration of the non-protein N_2, which was doubled after the extirpation (cf. following data).

Composition of saliva, mean figures	Non-protein N_2	Protein N_2	Total N_2	Reducing substance	D/N of proteins
Before extirpation (63 determinations)	11·5	62	73·5	142	2·29
After extirpation (26 determinations)	20·1	60	80·1	134	2·23

During exhaustion of the gland after extirpation the only difference found was the greater rise in the concentration of the non-protein N_2 as the secretion proceeded. Exp. 19 is a typical example of the exhaustion and recovery of the gland after the operation.

Exp. 19.

Time of collection	Saliva c.c.	Protein N_2 mgms. p.c.	Non-protein N_2 mgms. p.c.	Total N_2 mgms. p.c.	p.c. of exhaustion
29 mins.	30	60	20	80	0
5 hrs. 5 mins.	220	24	28	52	60
40 mins.	30	7	33	40	88
Recovery. Samples collected at intervals of 12 hours.					
36 mins.	30	26	27	53	57
31 "	30	46	20	66	23
39 "	30	61	20	81	0

Other experiments did not differ in the main points from Exp. 19. The recovery of the gland after extirpation was found in every case to be

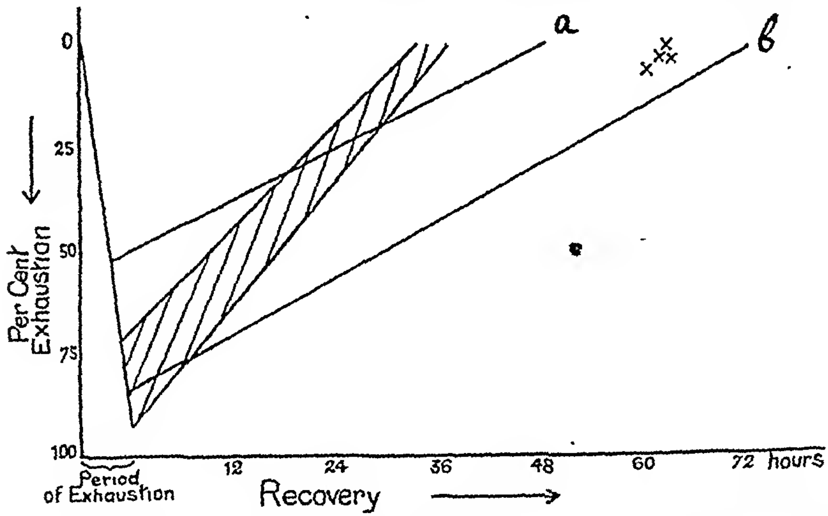

Fig. 1. Exhaustion and recovery of the gland as measured by the composition of the saliva. Space between *a* and *b*—cases of normal recovery; shaded area—cases of recovery after extirpation of the superior cervical sympathetic ganglion; *X*—the recovery under atropine: coincides with normal recovery.

quicker than before extirpation. We believe that both the increase in the non-protein N_2 and the quicker recovery can be best explained by a relatively larger supply of blood to the gland on account of section of the vaso-constrictor fibres.

The relative rates of recovery of the gland before and after extirpation of the ganglion are shown in the diagram.

Summary.

1. The recovery of an exhausted submaxillary gland has been studied under normal conditions.

2. The recovery is a slow process: it takes about three days for the gland to recover completely after a large secretion.

3. Paralysis of the secretory fibres of the chorda tympani by atropine does not influence the rate of recovery.

4. The recovery of the gland after extirpation of the superior cervical ganglion is accelerated.

5. An increase in the non-protein N_2 occurs after the extirpation of the ganglion.

6. The experiments performed do not support the theory that the sympathetic nerve has any "trophic" influence on the submaxillary gland under normal conditions.

The expenses of this research were defrayed by a grant from the Medical Research Council held by one of us (G. V. A.).

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THE NERVE FIBRE CONSTITUTION OF THE NERVES OF THE EYE. BY M. NAKANISHI.

(*From the Physiological Laboratory, Cambridge.*)

The short ciliary nerves.

It was shown by Bidder and Volkmann in 1842 that the nerve fibres running to and from the ciliary ganglion consist almost wholly of "small" nerve fibres. These small myelinated nerve fibres have generally been assumed to have no difference worth noticing from the small myelinated fibres of other parts of the autonomic nervous system. But recently Langley (1) has described the short ciliary nerves of the cat in the one specimen in which he measured them as consisting chiefly of fibres 4–4·5 μ in diameter, *i.e.* as being somewhat larger than the preganglionic fibres of the other parts of the autonomic system. In view of this, I have made measurements of the size of the ciliary nerves of the cat and sheep proximally and distally of the ciliary ganglion. The nerves were fixed in their normal length with 1 p.c. osmic acid and left in it for some hours. A portion was teased out in dilute glycerine; another portion was thoroughly washed, dehydrated, embedded in paraffin and cut. As a basis for comparison the cervical sympathetic of the cat from which the ciliary nerves were taken was in some cases treated with osmic acid in exactly the same way.

No difference was found in the size of the fibres in the short ciliary nerves of the cat and the sheep, notwithstanding the difference in the size of the animals. In the teased specimens all the fibres were between the limits of 3·6–4·5 μ; in the sections the great majority were also between 3·6–4·5 μ, but a few of 2·7 μ were present. As fibres of 2·7 μ were not found in the teased specimens, their presence in the sections may have been due to shrinking of some of the 3·6 μ fibres in dehydration and embedding in paraffin. In the 3rd nerve, a little proximally of the ciliary ganglion many fibres of 2·7–3·6 μ and a few of 1·8 μ are grouped together just under the perineurium, other scattered small fibres are 3–5 μ or 6 μ.

In the branch running to the ciliary ganglion the fibres were also nearly all 2·7–3·6 μ, only a few were 1·8 μ. In the cervical sympathetic of the cat, nearly all the fibres were 2·7–3·7 μ and there were a few

smaller ones, both in the sections and in the teased specimens. These three sets of fibres have very similar microscopical appearance. The results show that the preganglionic tectal fibres are of the same size as those of the sympathetic system, and that the postganglionic ciliary fibres are nearly all somewhat larger.

In looking up previous accounts I find hardly any distinct statements of the size of the fibres running to and from the ciliary ganglion. In the 3rd nerve the small nerve fibres are described by Gaskell(2) in the dog, by Barratt(3) in man and by Koch(4) in man and dog as being 3μ to 5μ or 6μ in diameter. Gaskell(2) says that the small fibres of the 3rd nerve run to the ciliary ganglion in the short root. This implies that the fibres of the short root are $3–5\mu$ in diameter, but Gaskell(2) also says that they are similar to those in the anterior roots of the thoracic nerves —the diameter of which he gave as $1{\cdot}8–3{\cdot}6\mu$—and that the fibres of the short ciliary nerves are all very small and of uniform size. Barratt(3) describes the short root of the ciliary ganglion as consisting of 3μ, with a few 9μ fibres and that the fibres of the short ciliary nerves are mostly $3–6\mu$. Koch(4) mentions that not all of the $3–6\mu$ fibres of the 3rd nerve pass to the ciliary ganglion.

Myelinated fibres of the muscle branches of the 3rd, 4th and 6th nerves.

It was noticed early in investigation on the size of nerve fibres that the anterior roots of the spinal nerves contained many large nerve fibres which were not found in the hypoglossal or in the muscle branches of the vagus. Gaskell(5) generalised the results, stating that the large motor nerves to visceral muscle were $7{\cdot}2–10{\cdot}8\mu$ in diameter and those to skeletal muscle were $14{\cdot}4–19\mu$ and upwards. Langley(1, p. 386) mentions that the fibres of $7{\cdot}5–11\mu$ though present are not numerous in the anterior roots of the spinal nerves. In the accounts of the ocular nerves by Gaskell and Barratt fibres between $6–11\mu$ are not mentioned as occurring, though this may only mean that relatively very few of this size were found. Koch(4) mentions a few fibres of medium size in the 4th nerve, but not in the 3rd and 6th nerves.

I have investigated the nerves to the external rectus, internal rectus and superior oblique muscles, *i.e.* a branch of the 3rd nerve, the 4th nerve and 6th nerve, in the cat and sheep. In all of these nerves I find fairly numerous fibres of $6{\cdot}5–10{\cdot}5\mu$ in diameter. I have roughly counted the intermediate fibres. I find them in the sheep to be about half as many as the larger fibres in all three nerves and in the cat to be in slightly greater proportion. I am unable, then, to confirm the previous accounts

as to the absence of fibres intermediate in size between the large and small.

Gaskell (2), Barratt (3) and Koch (4) class all fibres from 3μ to 5μ or 6μ as small fibres. Gaskell describes groups of such fibres as being present in the 3rd nerve and 4th nerve and a few such fibres, but not in groups, as being present in the 6th nerve. Barratt and Koch find no difference in the relative number of "small" fibres in the 4th and 6th nerves, in each they find that the ratio of small to large is about 1 : 3. Barratt finds the same ratio in the 3rd nerve, Koch a rather greater ratio. In the peripheral branches of all three nerves, I find no essential difference in the relative number of small fibres, in all the ratio is about 1 : 3.

Non-myelinated fibres in the ocular nerves.

The 6th cranial nerve has long been known to receive branches from the sympathetic (intercostal nerve) by way of the cavernous sinus. The 4th and 3rd nerves have also been said to receive sympathetic fibres from the plexus of the sinus, but some observers have failed to find any. My observations have been made on the non-myelinated nerve fibres in the nerve branches near the eye, in the sheep.

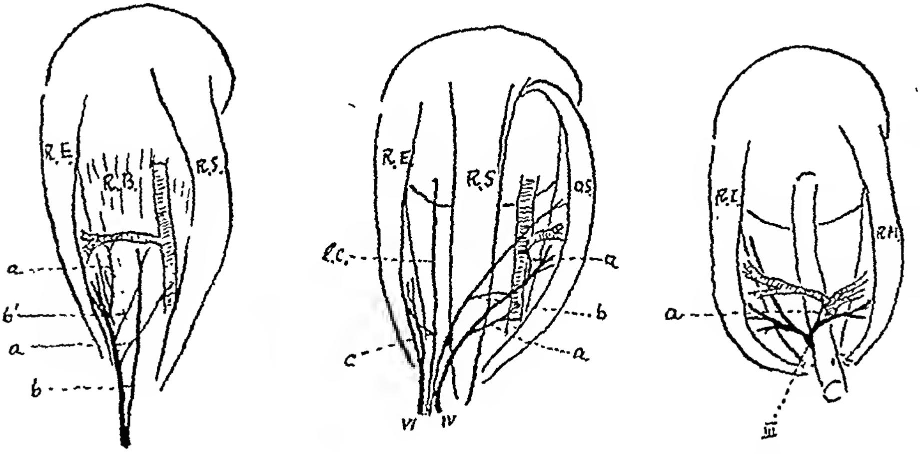

Fig. 1. Fig. 2. Fig. 3.

Fig. 1. *a*, fibres to vessels; *b*, branch of the 6th nerve to the retractor bulbi containing distinct non-myelinated fibres; *b'*, occasional non-myelinated strand from the 6th nerve to the retractor bulbi nerve.

Fig. 2. *a*, fibres to vessels; *b*, branch of the frontal nerve sometimes containing non-myelinated fibres; *c*, branch of the long ciliary nerve (*l.c.*) containing non-myelinated fibres.

Fig. 3. *a*, fibres to vessels.

The 6th nerve, as known, has a fairly conspicuous bundle of non-myelinated fibres: these lie just under the perineurium. The majority supply the unstriated muscle of the retractor bulbi (Fig. 1). The 6th nerve, distally of the branch to the retractor bulbi and the 4th and the 3rd nerves near their entrance into the muscles were teased out. In all specimens a few non-myelinated fibres were present either in small groups just under the perineurium or scattered in the perineurium.

From the 6th, 4th and the branch of the 3rd to the internal rectus muscle some filaments of non-myelinated fibres left the nerve to run to the neighbouring vessels (Figs. 1–3). Non-myelinated fibres also pass to the eye by all the long ciliary nerves. Most of them can be traced to the neighbouring vessels. In the distribution of the nerves at the periphery there are some variations. Thus a branch of a long ciliary nerve, containing non-myelinated fibres may join the 6th nerve. Sometimes the frontal nerve sends a filament containing non-myelinated fibres to the 4th nerve.

Summary.

Most of the postganglionic ciliary nerve fibres are somewhat larger (3·6–4·5 μ) than the preganglionic fibres (about 3 μ).

The nerves entering the ocular muscles have fibres of all sizes from 3 μ to about 17 μ. The relative number of the fibres of different size is not very different, but the 3–6 μ fibres are rather fewer than either the 6·5–10·5 μ or 11–17 μ fibres. The relative number of medium sized fibres is greater than in the anterior roots of the spinal nerves.

The greater part of the non-myelinated fibres of the 6th nerve run to the retractor bulbi; all the nerves entering the striated muscles have a few non-myelinated fibres; non-myelinated fibres also run to the eye by the long ciliary nerves: most of the non-myelinated fibres can be traced to blood vessels.

I am greatly indebted to Prof. Langley for his kind suggestion and advice during the course of the work.

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THE MAXIMUM REALISABLE WORK OF THE FLEXORS OF THE ELBOW. By T. E. HANSEN AND J. LINDHARD.

(From the Laboratory for the Physiology of Gymnastics, University of Copenhagen.)

In his paper on the maximum work of human muscles (1) A. V. Hill proposes "to adopt the term 'realisable maximum work,' and the symbol W, to mean the maximum work obtainable by any actual experimental means, from a contraction of any given duration; and the term 'theoretical maximum work,' and the symbol W_0, to mean the mechanical potential energy set free....". These terms have been adopted by Lupton in his first paper on this subject (2) and also by Hansen and Lindhard (3). The theoretical maximum can be determined by means of the stress-strain diagram as described by Hill in previous papers, and Hansen and Lindhard have devised a method, by the aid of which W_0 as defined above can be ascertained in man. Hill himself and Lupton have adopted another method. These two methods do not give consistent results, the first giving considerably higher figures than does the latter. Starting from the supposition that $W_0 = W +$ (the mechanical energy degraded in the rapid change of form), and, that this waste of energy is a simple function of the contraction time t, Hill determines W_0 as the asymptote to the curve obtained, when W is plotted against the "equivalent masses." The figures concerned are found to obey the equation $W = W_0 - k/t$ (1), or, in a more convenient form, $W = W_0 (1 - k/t)$ (4), where k is a constant depending on the internal friction of the muscle. Hansen and Lindhard assume, that the disagreement is due to a sort of fatigue coming on very soon, when contraction is maximal, and increasing with time, and Hill admits, that this supposition would explain the experimental facts, but he seems more disposed to seek for other causes: inertia of the dynamometer or some sort of nervous inhibition when working on the wheel (5). The first of these possibilities is, with regard to the construction of the dynamometer, quite out of question, and the second is *a priori* very improbable. The pull is, when determining W_0, performed in quite the same manner as

it is when determing W. Indeed, all the experimental facts brought forward lead to the assumption that the theoretical maximum work cannot be determined as done by Hill in his recent papers. To do away with the discrepancies by changing the definitions as done by Lupton (6) is no advantage. Lupton denotes W_0 as the maximum realisable work, but what then is W? Certainly, Lupton might define W_0 as the maximal "maximum realisable work," but then it still remains to show that this is equal to the "theoretical maximum" as defined by Hill. Hill and Lupton lay special stress on the fact that their results obtained on man agree with the results obtained by Hartree and Hill on frog's muscle and also with the theoretical considerations of Hill. But this fact does not justify their conclusions. It is well known that if we consider a short part of a curve only, it may approximately obey more than one equation; the discrepancies will become obvious, however, when the curve is continued. If the assumption of Hansen and Lindhard, that a steadily increasing fatigue-factor is at work in the determinations of W, is maintainable, it must be claimed that the curve obtained, when plotting W against the "equivalent masses," should not rise continually but at some or other definite value of the load bend down towards the abscissa, being zero, when the subject cannot turn the wheel at all. To test this point it would be desirable to have still larger "equivalent masses" than used even by Hansen and Lindhard, but the same goal can be reached when experimenting on subjects of inferior strength. It is of course a necessary condition that the subjects concerned are intelligent enough to be able to maintain a real maximal contraction throughout the pull; otherwise the results may be quite irregular.

We have with two young girls and an adult female subject obtained

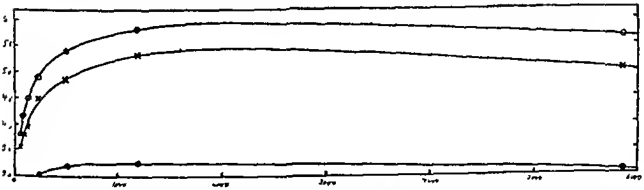

Fig. 1. Abscissæ = Eq. masses in kilos. Ordinates = work in kgm.-metres.

the results shown in Fig. 1, where W is plotted against the "equivalent masses."

The points determining the curves represent the averages of 5, 6 and

VARIATION OF CAPILLARY DIAMETER AND ANTIDROMIC ACTION IN THE FROG.

By SOROKU OINUMA.

(*From the Physiological Laboratory, Cambridge.*)

SINCE the date of Stricker's description of independent contractility in the capillaries of the excised nictitating membrane of the frog, capillary contractility has been affirmed by several investigators. A summary of the previous work has recently been given both by Krogh(1) and by Langley(2) so that I need not give it here. The conclusiveness of the evidence that a decrease of capillary diameter is due to active contraction is greatly dependent, in the ordinary conditions of the circulation, upon a knowledge of the degree to which the diameter varies with arterial pressure. It was to determine this question that my observations were begun; they have been extended to some other controversial questions.

Method. In all experiments the cerebral hemispheres were destroyed and a small dose of curari given. The capillaries were observed under a microscope with 200 or 400 magnification and ocular-micrometer. Observations on spontaneous variations in diameter were made, and on the same or following day the posterior roots of the lower nerves were prepared for stimulation, the cord cut above the 7th spinal root, and the part below excised. If the frog is kept cold and moist after such operation it may survive four to five days with good circulation. Observations were made both on spontaneous variations and on the effect of stimulating the posterior roots on more than ten frogs each of them allowing many series of observations.

Concurrent variations in the diameter of arteries and capillaries. The earlier observers took for granted that contraction of arteries in one region of the body would cause dilatation of both arteries and capillaries in the regions with uncontracted vessels. Doubt was thrown on the degree to which this occurred by the observation of Roy and Graham Brown(3) that extra-capillary pressure caused very little variation in the diameter of capillaries. From this and other results they concluded that the diameter of the capillaries depended mainly on their intrinsic tone. Instances of vascular dilatation caused by increased arterial pressure

were given by Langley (4), but he stated that increased rate of circulation was generally more obvious than dilatation of vessels. Krogh (5), as Roy and Graham Brown, came to the conclusion that capillary diameter was very little affected by arterial blood-pressure.

In the many observations I have made on spontaneous variations, I have found definitely independent contraction in two cases only and then only in two capillaries. In the first case, a part of a capillary contracted from 25 μ to 6 μ and a spindle-shaped bulge formed just centrally of it. Circulation ceased just after this contraction. In the second case a capillary of 19·2 μ diameter contracted to 13 μ. Circulation was good. This capillary was the last branch of the trunk of an arteriole, which had sent off many side branches. None of these side branches showed any contraction.

In the other cases, and in all the circulation was good, there was a variable increase or decrease of the diameter of capillaries but this always corresponded with increase or decrease of arteriole diameter. The following is a marked example of variation of capillary diameter.

Exp. 1.

Time of obs.	Arteriole diam. in μ	Percentage variation	Capillary diam. in μ	Percentage variation
3.51	33	—	13·2	—
3.53	33	—	13·2	—
3.55	46	+39	26·5	+100
3.58	40	−13	20·0	− 24·5

If there was no variation in the diameter of the arterioles, then the capillaries showed no variation of their size.

In order to determine further the effect of variation of blood-pressure on capillary diameter, experiments were made in two ways:

(*a*) A loose thread was passed round the femoral artery, or the abdominal aorta, and the circulation through the artery was stopped by pulling up the thread. After compression of the femoral artery a very slow circulation usually remained in the web. If the circulation before compression was very good, there was, on compression, a slight diminution of the diameter of capillaries which returned to the former size when blood was allowed to flow again through the artery. The diminution was, however, usually less than that observed in the spontaneous variations.

Exp. 2.

	Size of capillary in μ (*a*)	Size of capillary in μ (*b*)
Before compression	23 0	19·8
Artery compressed	19·8 (−14 %)	13·2 (−33 %)
Compression off	23·0	26 4

(*b*) The middle of the thigh was gradually compressed with the finger. By this means the vein was closed without closing the artery. The calibre both of arteries and capillaries increased.

Exp. 3.

	Size of arteriole	Size of capillaries (*a*)	Size of capillaries (*b*)
Before compression	—	25·0	16·0
Vein compressed	—	37·5	22·5
		(c)	
Before compression	38·5	15·0	
Vein compressed	48·5	19·2	

With regard to the compression experiment it must be remembered that arterioles and capillaries dilate as the result of cutting off the supply of blood. In one of my experiments with compression of the abdominal aorta in which the circulation in the web completely stopped, the dilatation of arterioles and capillaries began 1 min. after compression and attained a maximum after 2 mins.

The results given above show, I think, that, as a rule, in the given conditions, arteriole variation is accompanied by capillary variation of the same sign, *i.e.* that, as a rule, the diameter of the capillaries varies with the blood-pressure. A given arteriole variation in diameter, is however, accompanied in different conditions by different degrees of capillary change. In fact, as I have to mention presently, I have not found any distinct dilatation of capillaries to accompany the arteriole dilatation produced by posterior root stimulation, and sometimes there was no decrease of capillary size during reflex arterial contraction, and further it is known that complete occlusion of the arteries does not cause collapse of the capillaries.

Effect of brushing the web. Krogh (6) found contraction of part of a capillary on local mechanical stimulation. This local effect I have not investigated, but I made some observations on the effect of brushing the web with a brush moistened with Ringer's fluid. This readily caused strong contraction of the arterioles. The capillaries decreased somewhat in size, but the decrease was slight and was equal in the whole area of the capillaries observed. No evidence of independent capillary contractility was obtained. The arteriole contraction was not reflex, for it was obtained after section of the sciatic. It was apparently caused by direct stimulation of the muscle coat, for it was obtained after applying cocaine to the web. I conclude that mechanical stimulation of moderate strength does not directly affect capillary diameter.

Innervation of capillaries. Steinach and Kahn (7) found that some

capillaries in the frog's nictitating membrane can be brought to complete obliteration by stimulation of sympathetic fibres. Krogh and his co-workers (8) found also that stimulation of the lower ganglion of the sympathetic chain brings about a constriction first of arteries and a few seconds later also of the capillaries of the web. They believe that every Rouget cell which surrounds a capillary is supplied by a sympathetic nerve fibre capable of causing contraction of the cell and so constriction of the capillary. I investigated the question in the following ways.

After cutting both roots of the 7th, 8th and 9th spinal nerves close to the cord, the spinal cord was cut just above the level of the 7th spinal nerve and the part caudal of the section was excised. Then, observing the web under the microscope, I pinched the flank of the same side. Reflex arterial contraction was always produced but the capillaries were usually only slightly affected and sometimes not at all.

		Size of arteriole	Size of capillary
Exp. 4.	Before pinching	46·0 μ	12·8 μ
	Pinching of flank	Contract	9·6
Exp. 5.	Before pinching	53·0	13·2
	Pinching of flank	39·6	13·2

In these cases the nervous impulses causing vaso-constriction leave the cord by the upper rami communicantes, pass down the sympathetic chain and reach the sciatic nerve by the lower rami communicantes.

I tried also direct electrical stimulation of the upper part of the cord after removal of the lower part; it caused greater contraction of arterioles than that produced reflexly. In one experiment out of ten local constriction occurred in a few capillaries (see Exp. 6).

Exp. 6.

	Size of capillaries	
	(*a*)	(*b*)
Before stimulation	9·6 μ	16 0 μ
Spinal cord stimulated	3·2 (− 67 %)	9·6 (− 40 %)
After stimulation	—	22·4

In other cases there were only variations concomitant with that of the arterioles. These variations did not take place at a particular point of a capillary, as they did in Exp. 6, but occurred along its whole length. The following two examples show the usual range of variation.

Exp. 7.

	Size of capillaries	
	(*a*)	(*b*)
Before stimulation	19·8 μ	12·8 μ
Stimulation of spinal cord	13·2 (− 33 %)	9·6 (− 25 %)

Stimulation of posterior roots. Doi (9) found dilatation of arterioles as well as of capillaries in the web on stimulating the posterior roots. After dilating the arterioles by injecting acetyl-cholin the posterior roots caused dilatation of the capillaries only, and Doi concluded that the dilatation of the capillaries was due to a direct effect upon them. Krogh and his co-workers (8) confirmed this result. Twelve years ago I tried the antidromic action of posterior roots on the frog's web without success. Possibly the failure was due to the outspread of the stimulating current to the spinal cord. In my present experiments, stimulation of the posterior roots on frogs from which the hinder half of the spinal cord had been excised, always caused distinct dilatation of arterioles. I did not find any dilatation of capillaries, but it happened that in the experiments in which the arteriole dilatation was most marked, my attention was given to this and not to the effect on the capillaries. Antidromic action on capillaries was also absent so far as I could see in the experiments to be mentioned presently on the skin of the back and on the tongue.

Effect of some drugs upon capillaries. Krogh (6) describes the application of 0·1 p.c. adrenaline to the tongue of the green frog as causing capillary dilatation accompanied usually by dilatation of most of the smaller arteries. In the web, he found (1, p. 136) that adrenalin had no effect on the capillaries. In my experiments the application of 0·1 p.c. adrenaline to the frog's web caused such distinct contraction of arterioles that they sometimes closed completely and if the adrenaline was not washed off, its action continued for a very long time. The capillaries, on the contrary, dilate. There is, however, a preliminary contraction which may be due to the fall of blood-pressure by the contraction of arterioles. Capillary dilatation occurs usually during contraction of arterioles, and as a result of the contraction a dilated capillary is sometimes quite free from blood corpuscles, and sometimes congested further by blood streaming back from the vein. The result shows that if there are sympathetic constrictor nerve endings in the capillaries, adrenaline does not stimulate them.

Exp. 8.

	Diameter of capillaries in μ		
	(*a*)	(*b*)	(*c*)
Before application	19·8	26·5	19·8
Adrenaline applied	26·5	39·5	26·5
Blood streaming back from vein	33·0	—	—

Application of 0·1 p.c. histamin to the web had no effect either on arterioles or capillaries. Krogh (6) describes also inactivity of histamin on the blood-vessels of the frog's web.

It is well known that local application of mustard oil causes distinct dilatation of arterioles and capillaries I confirmed this on the frog's web and found an exact correspondence between the variation in the capillaries and that in the arterioles

Exp 9

Time of observation	Size in μ of Arteriole	Size in μ of Capillary
3 50	39 5	19 8
3 51*	33 0	13 2
3 53	33 0	13 2
3 55	46 0	26 5
3 58	40 0	20 0

* One drop of mustard oil on the web

Mechanism of antidromic action on arterioles As I have said above, I always found in this series of experiments that dilatation of arterioles was caused by stimulation of the peripheral end of the posterior roots The extent of the dilatation is illustrated in Exps 10 and 11

	Diameter of arterioles in μ (a)	(b)	(c)
Exp 10			
Before stimulation	46 0	19 8	19 8
Stimulation of 8th posterior roots	53 0 (+15 %)	26 5 (+22 %)	33 0 (+40 %)
After stimulation	46 0	19 8	26 4
Exp 11	(a)	(b)	(c)
Before stimulation	19 8	13 2	22 5
Stimulation of 8th and 9th posterior roots	33 0 (+40 %)	19 8 (+33 %)	32 0 (+29 5 %)
After stimulation	19 8	13 2	22 5

As these figures show, the maximal increase of diameter was 40 p c, the minimum 15 p c, the average of eight cases was 28 p c The response begins, in good condition of the frog, in 10 seconds from the beginning of a faradisation lasting 15 to 30 seconds, and reaches its maximum during stimulation If the animal is not very excitable, the response appears one to two minutes after cessation of stimulation The currents were of a strength which caused a distinct pricking sensation on the tip of the tongue Cooling of the web with ice prolongs the latent period of response In one experiment with cooled web, the dilatation began in 10 seconds and reached its maximum 20 seconds after cessation of the stimulus

The use of curari prevented recognition of escape of current to the anterior roots The anterior roots of the 9th and sometimes those of the 8th nerve cause contraction of the bladder and cloaca Although it is not very probable, it is conceivable that the contraction by compressing the blood-vessels might raise the blood pressure, and the raised blood

pressure distend the arteries of the web. In consequence, experiments were made to test this possibility.

The sciatic nerve on one side was exposed in the middle of the thigh and a small sheet of india-rubber placed under it. After testing the vaso-dilatation by stimulating the posterior roots, the part of the sciatic nerve on the rubber sheet was wrapped round with a small piece of cotton wool soaked in novocaine solution. The completeness of the paralysis of the nerve was tested by the disappearance of vaso-constriction on pinching the flank or on stimulation of the spinal cord. Complete paralysis was usually obtained 15 minutes after the novocaine application. Then the effect of stimulating the posterior roots was tested on two or three different arterioles. After this, the cotton wool with novocaine was removed, the sciatic nerve washed with Ringer solution and wrapped in cotton wool with Ringer. When the conductivity of the nerve had returned the effect of the posterior roots was tested again.

The experiments were decisive. Abolition of the conductivity of the sciatic abolished the vaso-dilator effect of the posterior roots. In one case only, a slight dilatation—from 56 μ to 59 μ—was obtained. Thus the effect is not a passive effect brought about by constriction elsewhere, but is a direct effect of posterior root fibres on the arterioles. Examples of the results obtained in three frogs are given in Exps. 12 to 15.

		Diameter of capillaries in μ		
		Before application of novocaine	During paralysis by novocaine	After recovery
Exp. 12.	Before stimulation	52·8	59·3	52·8
	Stimulation of posterior roots	66·0	59·3	59·3
Exp. 13.	Before stimulation	19·8	39·6	26·4
	Stimulation of posterior roots	39·6	39·6	36·3
Exp. 14.	Before stimulation	39·6	33·0	16·5
	Stimulation of posterior roots	43·0	33·0	26·4
Exp. 15.	Before stimulation	66·0	79·0	59·2
	Stimulation of posterior roots	79·0	79 0	72·5

Another form of experiment was tried. The femoral artery was clamped and the posterior roots then stimulated. The stimulation in some cases, but not constantly, caused dilatation of the arterioles. The results in such experiments are complicated by the effect of anæmia.

In the course of these experiments, I found that pinching the skin of the flank during the abolition of sciatic conductivity by novocaine usually caused distinct dilatation of the arterioles, thus giving evidence in addition to that already given of passive dilatation. On recovery of

sciatic conductivity, pinching caused the normal vaso constriction. Pinching the skin also caused dilatation instead of constriction of arterioles when the sympathetic trunk was cut just above the 8th ganglion. Examples of these effects are given in Exps. 16 and 17.

Exp. 16

	Diameter of arterioles in μ		
	Before narcosis	During paralysis	After recovery
Before pinching	39.5	36.4	33.0
Pinching of flank	26.2	46.2	26.4

Exp. 17

	Before cutting of sympathetic trunk	After cutting of sympathetic trunk
Before pinching	19.8	19.8
Pinching of flank	13.2	26.4

Effect of stimulating the nerves of the dorsal skin. Langley (4) found that stimulation of a cutaneous nerve to any part of the skin caused vaso constriction in the part adjoining the anatomical ending of the nerve in it and that successive branches of the dorsal cutaneous nerves supplied successive areas which overlapped slightly. I have made 15 observations on nine frogs, stimulating the peripheral ends of the dorsal cutaneous nerves after curarisation. In three cases I found no effect, in two cases contraction of arterioles, and in ten cases dilatation of arterioles. It may be assumed that the dilatation is due to antidromic action, it appears then that the balance of effect of the sympathetic vaso constriction and of the posterior root vaso dilatation turns in one direction or the other according to yet undetermined body conditions. I reproduce some examples of dilatation and constriction.

Exp. 18

	Diameter of arterioles in μ				
	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Before stimulation	75.0	106.0	39.5	26.4	50.0
Stimulation of cutaneous nerve	87.5	119.0	46.2	33.0	37.5
After stimulation	75.0	106.0	—	29.7	50.0

Effect of stimulating the glossopharyngeal nerve. Dilatation on stimulating the peripheral end of the glossopharyngeal nerve in the frog was first described by Lépine (10). He found that the stimulation caused secretion and flushing of the corresponding half of the tongue. Krogh (1) obtained dilatation on mechanical stimulation of the nerve and observed that it occurred both in the capillaries and in the arteries. It hardly, then, needs further confirmation. I have, however, made some observations on the degree of dilatation which is caused by faradic stimulation of the glossopharyngeal. The increase of diameter ranged from 8.2 p.c. to 64 p.c.

SUMMARY.

The results, unless otherwise mentioned, refer to the vessels of the frog's web.

1. Whilst, as is known, complete contraction of arterioles does not cause collapse of capillaries, decrease or increase of arteriole size is, as a rule, accompanied by decrease or increase of capillary size. The frequency of exceptions to this rule vary in different conditions. Exceptions were not infrequent in reflex contraction of the arterioles, and posterior root stimulation, though it caused arteriole dilatation, was not found to cause appreciable dilatation of capillaries. Further, the degree of capillary variation caused by a given arteriole variation is not constant.

2. It is concluded that capillary size depends partly on the blood-pressure and partly on the condition of the capillaries.

3. Evidence of independent capillary contractility, *i.e.* constriction of a *portion* of a capillary, was rarely obtained (*a*) in spontaneous variations, (*b*) when arteriole contraction was produced reflexly or by stimulating the sympathetic origins in the upper part of the spinal cord or by brushing the web.

4. Adrenaline causes contraction of arteries and (as described by Krogh in the tongue), dilatation of capillaries.

5. Novocaine applied to the sciatic nerve prevents dilatation being produced by posterior root stimulation, but does not prevent dilatation (presumably passive) being produced reflexly by skin stimulation.

6. Stimulation of the dorsal cutaneous nerve was found to cause arterial dilatation more frequently than arterial contraction.

In conclusion I have to record my grateful thanks for the suggestions and kind aid which I received from Prof. Langley.

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ON THE BLOOD PHOSPHATE AFTER INSULIN CONVULSIONS. By L. B. WINTER and W. SMITH

(*Beit Memorial Research Fellows*).

(*From the Biochemical Laboratory, Cambridge.*)

In a recent paper (1) it was shown that the inorganic blood phosphate is diminished as the result of injecting insulin into rabbits. Perlyzweig, Latham and Keefer (2) and Harrop and Benedict (3) have also shown this to be the case. The latter showed in addition that this fall occurs in the treatment of diabetes mellitus in man by injection of insulin.

It has been shown that animals may be recovered from insulin convulsions by means of glandular extracts. Since no abnormal amounts of reducing sugar are excreted as a result of insulin administration, and in view of the work of Dudley and Marrian (4), who showed that glycogen was almost entirely absent from the liver and muscles of mice after insulin convulsions, the recovery of animals from convulsions by means other than injection of glucose, would make it probable that the sugar stored as glycogen has been converted into some other form. It is unlikely that the animal has burnt all its available carbohydrate when the convulsion point is reached (5). Magnus-Levy (6) postulated acetaldehyde as a cleavage product of carbohydrates in the body. It is of great interest that Neuberg and others (7) have recently found that the production of acetaldehyde by the liver *in vitro* is greatly increased in the presence of insulin. It may be that the injection of insulin into normal animals sufficient to cause convulsions leads to the conversion of the sugar stored as glycogen into some intermediate product of which the further metabolism on normal lines is prevented by the excess of insulin still present, the result being that carbohydrate metabolism is in abeyance until this excess is neutralised, or sugar is actually added to the body as by injection of glucose. If the fall in the blood sugar and inorganic phosphate after injection of insulin had been due to the formation of a sugar-phosphate complex it would appear probable that during the process of recovery by gland extracts the blood sugar would rise along with the inorganic phosphate. A complication may arise owing to the possibility of different animals going into convulsions when they have not lost all their glycogen. Insulin may have a nervous effect as well as

one directly acting on the carbohydrate metabolism (8). The following is an account of work undertaken to determine the alterations in the values of the inorganic blood phosphate after recovery from insulin convulsions by different means.

Experimental. Rabbits were used throughout. Blood sugar estimations were made by Bang's old method, inorganic blood phosphate by that of Bell and Doisy (9) as modified by Briggs (10). It has been pointed out by Lehman (11) that in the case of rabbit blood a yellow colour often interferes with the blue by which comparison is made in the above method for determination of phosphate. With several animals we have found this to be the case, and conclusions based on these experiments may lead to serious error. In the majority of cases no such difficulty was experienced. The samples of blood obtained during and after convulsions were as a rule taken from the heart, owing to the difficulty of obtaining sufficient blood from the ear during the state of shock. Recovery after injection of glucose was first studied. The blood phosphate in all cases only rose slowly to its original value, in many cases a further fall occurred, and the normal value had not been reached 24 hours later. When glucose is used for recovery it is probable that the sugar injected will be dealt with along two lines. Some of the sugar will suffer the fate of the animal's own carbohydrate due to the excess of insulin still present in the body; the rest, if sufficient has been injected, will be available for restoring the blood sugar to a normal level and for utilisation. This view is supported by the fact that an injection of glucose may only temporarily relieve the animal, a second injection being necessary some time later in order to combat further convulsions. This is well shown in the case of rabbit 14 in the previous paper in which the blood phosphate was lowest at the time of the second convulsion. When the amount of glucose used for recovering rabbits from convulsions was injected into normal rabbits no appreciable alteration in the inorganic phosphate occurred.

Recovery from convulsions by means of adrenaline was next studied. Controls were performed in which adrenaline alone was injected intravenously into rabbits. The results showed that a fall in the inorganic phosphate occurred, during which time the blood sugar rose above normal. This has also been shown by Perlyzweig, Latham and Keefer (2). A possible explanation of this is that an additional supply of sugar is present which has to be disposed of again to restore the blood sugar to a normal level. This process may necessitate the interaction of inorganic phosphorus. When recovery by means of adrenaline was attempted it was found advisable to inject two small amounts with an interval of time

between rather than the whole amount at the same time. It is easy to kill a normal rabbit by injecting adrenaline in amounts greater than 0·4 c.c. of 1 : 1000 solution for a rabbit of 2 kilos. The shock of the insulin convulsions renders rabbits very sensitive to injection of such substances as pituitrin or adrenaline. With ether extract the animals sometimes died shortly after the injection. Usually, good recovery ensued, but sometimes the animals appeared abnormal or even convulsed some hours later and a further injection was necessary to ensure permanent recovery. After recovery by adrenaline the inorganic phosphate as a rule at first rose along with the blood sugar. Later, however, the value for the phosphate fell whereas the blood sugar had reached a height usually above normal. Evidently some secondary adjustment was taking place, but the initial rise after convulsions when adrenaline is injected is in marked contrast to the fall which occurs when adrenaline is injected into a normal animal. We have found (unpublished experiments) that injection of adrenaline in similar amount into normal rabbits alters the nature of the blood sugar in that when a comparison was made between the observed optical rotation (P) and that calculated from the reducing power of the carbohydrate on the basis that glucose is the only reducing substance present (the latter factor is referred to as C) it was found that the value of P was greater than that of C. In contrast with this the value of P is less than that of C in the case of the normal animal. This increased amount of sugar present in the blood after injection of adrenaline being similar to that of diabetics as evidenced by the ratio P/C would appear to be an abnormal product and necessitate further change before it can be converted into a normal blood sugar. In an attempt to determine whether the blood sugar of a rabbit could be increased in amount and yet be unaltered as regards its ratio P/C we injected thyroid extract previous to the injection of adrenaline. It was then found that while the blood sugar was raised to a degree comparable with that caused by adrenaline alone, the ratio P/C was as a rule within the limits normally met with in the rabbit, P being less than C. For this reason in some experiments thyroid extract was injected before adrenaline for the recovery of rabbits from insulin convulsions. In some samples of thyroid tablets traces of parathyroid were evidently present since the convulsions were greatly increased with the resultant death of the animal. It has been shown (12) that parathyroid extract intensifies the action of insulin. Other samples of thyroid powder free from parathyroid were used. The ease of recovery did not appear to be increased and the behaviour of the phosphate was not different from that when adrenaline alone was used.

We found that when an extract of fresh thyroid gland from which the parathyroids had been dissected out was injected into rabbits along with a normal dose of insulin, the action of the insulin was unaltered as shown from determinations of the blood sugar. Samples of commercial thyroid powder were therefore tested by injection together with insulin. When the action of the insulin was not increased, it was concluded that the sample was free from admixture with parathyroid. Armour's thyroid siccum has been found to be apparently uniform in that it caused no intensification of the action of insulin.

In order to determine whether thyroxin was of value in the recovery of animals from insulin convulsions, a sample was obtained from Messrs Squibb and Sons, New York. We were unable to recover rabbits by means of thyroxin alone, and even when it was injected along with adrenaline considerable difficulty was experienced in effecting recovery. Thyroxin alone had only a small effect on the inorganic blood phosphate.

Since recovery was so difficult after thyroxin had been injected into the animal, it was of interest to determine the ratio P/C after injecting thyroxin and adrenaline into normal rabbits. In every case the value of P exceeded that of C. It would appear likely that there may be some other active principle present in dried thyroid powder which has the effect of interacting with adrenaline for the formation of normal blood sugar. That this is not due to traces of parathyroid is clear because parathyroid extract and adrenaline when injected together behave as adrenaline alone, P being greater than C.

Bodansky (13) has studied the effect of thyroxin on the recovery of the blood sugar after insulin. Sheep were used, a dose of insulin being given which caused a marked fall in the blood sugar though it was not great enough to cause convulsions. When thyroxin was injected at a time when the blood sugar had begun to rise again, the blood sugar rose more rapidly and to a higher level. In a later communication (14) he showed that the recovery of the blood sugar after similar doses of insulin was delayed in the case of sheep from which the thyroids had been removed. He assumes that the action of thyroxin in recovery after insulin is to break down glycogen from the liver. In view of the work of Dudley and Marrian (4) this is unlikely, and in the light of the experiments on recovery from convulsions recorded above, it is probable that some constituent of the thyroid other than thyroxin is also necessary.

Since injection of pituitrin has no effect on the blood sugar of the normal animal, the mechanism by which it causes recovery from insulin convulsions is obscure. Dale (5) has suggested that it is directly an-

tagonistic to insulin; it is possihle therefore that the injected pituitrin neutralises the excess of insulin still present and allows the enzymes responsihle for the breaking down of the sugar stores to hecome operative and raise the blood sugar to a normal level. It became of interest to determine the variation in the value for the inorganic phosphate under these conditions. Considerahle difficulty was sometimes experienced in recovering the animals from convulsions by pituitrin alone. In the case of several rabbits the first injection intensified the convulsions and death occurred in a few minutes. Dudley has shown (15) that there are at least three active principles in the pituitary, and since it is uncertain which is at work in recovery from convulsions, the method of standardisation, viz. a determination of the effect on plain muscle, may result in a product of varying efficacy with regard to the content of the active principle which is antagonistic to insulin. When the animals made a good recovery as a result of injecting pituitrin, the hehaviour of the blood sugar and inorganic phosphate was extremely variahle. In some animals the blood sugar might rise only slightly and the value for the phosphate he high, the animal meanwhile appearing normal. In other experiments the value for the phosphate was lower or unaltered after recovery, while the blood sugar rose rapidly to normal. From these experiments little light can be thrown on the mechanism of recovery hy pituitrin. It is difficult to suppose that the hlood sugar which is formed in recovery from convulsions by means of pituitrin comes from a source different from that which gives rise to sugar under the influence of adrenaline, or adrenaline and thyroid extract. But the behaviour of the inorganic phosphate is different in the two cases. Since the value foя the inorganic phosphate may remain unaltered at a low level, while the hlood sugar has returned to normal, it may be evidence against the conversion of glycogen to a sugar-phosphoric acid complex as a result of insulin convulsions.

SUMMARY.

1. Variations in the inorganic blood phosphate after insulin convulsions in rahbits have been studied.

2. Rabbits have been recovered from convulsions by means of glucose, adrenaline, adrenaline and thyroid extract together, and pituitary extract.

3. After adrenaline the inorganic phosphate usually regains the normal level quickly, but falls again. After pituitrin the effect is variable. After glucose the normal value is not regained for a considerable period.

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PROTOCOLS.

The following are typical experiments. All were on rabbits.

Glucose

	Time	Blood sugar	Inorganic phosphate as mg. P %
A. 2 kg.	10.00	·10 %	6·20
	10.10	35 c.c. 10 % glucose	—
	11.25	·20 %	6·15
	2.30	·12 %	6·30
B. 2 kg.	10.10	·11 %	6·25
	10.20	45 mg. insulin	—
	12.30	Convulsions ·05 %	5·10
	12.40	Glucose	—
	2.30	·09 %	3·95
	4.30	·12 %	3·70
	Next day	·11 %	4·00
	Five days later	·10 %	5·95

Adrenaline

Adrenaline (*contd*)

	Time	Blood sugar	Inorganic phosphate as mg P %
E 2 8 kg	9 40	12 %	5 50
	10 00	50 mg insulin	—
	12 15	Convulsions 06 %	2 40
	12 25	0 3 c c adrenaline	—
	12 30	0 2 c c adrenaline	—
	12 45	Animal appeared normal	—
	3 00	·11 %	4 75
	5 50	12 %	4 20

All the blood samples were obtained from the ear

E Same animal 6 weeks later Samples obtained from the heart

	Time	Blood sugar	Inorganic phosphate as mg P %
	9 45	13 %	4 60
	10 00	20 mg insulin	—
	10 03	15 mg parathyroid (Armour)	—
	1 30	Convulsions 06 %	2 60
	1 45	1 7 c c adrenaline subcut	—
	1 55	Animal eating	—
	2 30	Convulsions 1 c c adrenaline subcut	—
	3 45	07 %	5 10
	8 30	14 %	3 80

Adrenaline and thyroid

	Time	Blood sugar	Inorganic phosphate as mg P %
F 2 5 kg	10 00	12 %	5 50
	10 25	40 mg insulin	—
	12 40	Convulsions 05 %	4 70
	12 50	100 mg thyroid sicc (Armour)	—
	12 55	0 5 c c adrenaline	—

Adrenaline and thyroid (*contd*)

	Time	Blood sugar	Inorganic phosphate as mg P %
F 2 5 kg	1 10	Animal eating	—
	2 50	13 %	5 40
	5 00	12 %	5 55
	Next day	13 %	5 35

Pituitrin

	Time	Blood sugar	Inorganic phosphate as mg P %
G 2 3 kg	9 45	11 %	5 15
	10 00	2 c c pituitrin	—
	12 00	11 %	5 70
	2 45	11 %	5 50
H 1 8 kg	10 10	10 %	4 90
	10 15	35 mg insulin	—
	12 15	Convulsions 04 %	3 60
	12 20	3 c c pituitrin	—
	12 25	Eating	—
	2 50	06 %	2 65
	3 40	Weak, 1 c c pituitrin	—
	4 45	12 % Eating	3 80
	8 55	11 %	2 60
	Next day	11 %	3 25
I 2 5 kg	9 45	12 %	3 75
	10 00	50 mg insulin	—
	3 00	Convulsions 04 %	2 50
	3 05	2 c c pituitrin	—
	3 10	Eating	—
	4 20	Weak, 2 c c pituitrin	—
	5 15	06 %	3 00
	8 15	06 %	5 10
	9 15	Eating well	—
	Next day	12 %	3 70

	Time	Blood sugar	
J 2 7 kg	10 00	11 %	
	10 15	1 mg thyroxin	
	10 35	0 4 c c adrenaline	
	10 50 (killed)	30 %	a for $l = 1$
		35 c c blood	C = +0 10
			P = +0 12
K 2 5 kg	10 05	11 %	
	10 15	1 mg thyroxin	
	10 30	0 4 c c adrenaline	
	10 45 (killed)	28 %	
		41 c c blood	C = +0 11
			P = +0 17
L 3 2 kg	10 10	10 %	
	10 15	80 mg thyr sicc (Armour)	
	10 20	0 3 c c adrenaline	
	10 35 (killed)	18 %	
		55 c c blood	C = +0 15
			P = +0 10
M 2 5 kg	10 15	11 %	
	10 20	30 mg parathyroid powder	
	10 35	0 3 c c adrenaline	
	10 50 (killed)	19 %	
		45 c c blood	C = +0 11
			P = +0 16

THE EFFECT OF FATIGUE ON THE RELATION BETWEEN WORK AND SPEED, IN THE CONTRACTION OF HUMAN ARM MUSCLES. By A. V. HILL, C. N. H. LONG and H. LUPTON.

(*From the Department of Physiology, University College, London.*)

In recent papers A. V. Hill(1) and Lupton(2) have shown that in a maximal contraction of the flexor muscles of the elbow the work done is related to the time occupied in doing it by an equation of the type $W = W_0(1 - k/t)$. The dependence of work done upon speed of contraction is being further investigated by Prof. Gasser here, who finds the same phenomenon to occur in the case of isolated frog's muscle subjected to a maximal tetanus: one is obviously dealing with a fundamental characteristic of muscular tissue and not merely with a property of its innervation. Some doubt has been thrown by Hansen and Lindhard(3) upon the use of the equation to describe the phenomena: their objections, however, have been shown by Hill(2) to reduce to the fact that the constant W_0 determined from the observations relating W to t is some 10 p.c. to 20 p.c. less than the theoretical maximum work calculated from an "indicator" diagram relating force exerted to amount of shortening.

The cause of this divergence is not explained. It is small and has no influence on the essential fact that the work increases with duration of shortening: it makes the real "theoretical maximum work" slightly greater than W_0.

In a paper appearing in the present number of this *Journal*, and very courteously communicated to us before publication, Hansen and Lindhard show that fatigue appreciably diminishes the value of W observed, when the duration of shortening is prolonged. In order to obtain sufficiently long times on the wheel at their disposal they employed subjects of "inferior strength." This method undoubtedly shows qualitatively the incidence of fatigue in prolonged pulls by such subjects, but we felt it advisable to make a direct quantitative estimate of the effect of fatigue upon the work done by the vigorous male subjects with whom most of our experiments have been made. The following method was employed.

A given pulley of the wheel being chosen the subject made a series of maximal contractions (usually about 25 in number) employing the quick release mechanism previously described On the signal "go" he made, and maintained, a maximal effort, and after an interval accurately measured on a stop watch the wheel was released The interval was varied arbitrarily between 0 and 3 secs, the subject having no idea beforehand what it would be in any given contraction In this way the same identical movement was carried out in all about 25 times, preceded by an interval of maximal isometric contraction varying up to 3 secs Plotting the work against the duration of the isometric interval we can obtain an estimate of the effect of fatigue The experiment was made on a number of healthy male subjects, on various pulleys (*i e* against various equivalent masses), and starting either with the arm completely extended, or bent at an angle of 40° No consistent differences (for any given subject) were found, either for different pulleys, or for different positions of the arm in the initial isometric contraction The results obtained from each group of about 25 observations on a given subject were expressed in terms of the percentage diminution in the work done, resulting from each 1 sec of preliminary maximal contraction Altogether about 1000 observations were made, so enabling a fairly accurate estimate to be obtained of the effect of fatigue

TABLE *Per cent. reduction in the work done resulting from each 1 second of preliminary maximal contraction*

[Each number given is the mean value obtained from a series of about 25 observations]

Downing	6 8	7 2	6 0	4 0	12 0	10 4	Mean 7 8
Parkinson	6 4	6 0	—	—	—	—	„ 6 2
Lupton	3 6	3 0	7 0	7 8	—	—	, 5 4
Long	7 0	8 0	4 0	9 6	6 0	9 6	„ 7 4
Liljestrand	7 4	5 0	—	—	—	—	, 6 2
Azuma	7 6	6 6	6 0	6 4	4 2	—	„ 6 0
Gasser	0	3 6	2 0	0	3 2	—	, 1 8
Weakley	4 0	7 0	—	—	—	—	„ 6 5
Scheinfein	6 6	5 2	5 4	9 8	—	—	„ 6 8

The individual subjects varied somewhat from one another, but a 6 p c reduction is a good mean estimate for the effect on the work of each 1 sec of previous exercise Thus if 10 kg m of work be done in an undelayed contraction, about 9 4 kg m will be done if shortening be delayed for 1 sec after the signal "go" Clearly A V Hill (2, p 352) was in error in stating that no appreciable diminution in the work results from a second or two *delay in the release*, though this appears to be the case with himself and Gasser We must consider the effect of this diminution on the form of the relation between W and t

The best data available are those given by Lupton(2, p. 72), from which we obtain:

Corrected work	10·51	9·76	9·15	7·72	6·67	5·76	4·66
Calculated work	10·24	9·69	9·11	7·75	6·71	5·71	4·55

The "corrected work" is calculated as follows. It is assumed that in any given contraction every element in the work done by the shortening muscle is reduced 6 p.c. by each previous 1 sec. of maximal contraction, and proportionately for other durations. If the shortening occupy t secs. and be uniformly accelerated (as Hill(1) previously showed to be approximately the case) the total reduction can then be shown to be 6 p.c. $\times$ $2/3t$, *i.e.* $4t$ p.c. If we employ the formula $W = 11{\cdot}78\ (1 - {\cdot}283/t)$ we obtain the numbers given in the last row for "calculated work," which clearly agree closely with those given for "corrected work." The allowance for fatigue, therefore, does not alter the general character of the curve, or the accuracy with which the observations may be expressed by the equation. Lupton gave 10·96 and 0·264 as the values of W_0 and k determined from the observations uncorrected for fatigue: the values 11·78 and 0·283 imply that the true "viscosity" coefficient $k = 0{\cdot}283$ and the true theoretical maximum work $W_0 = 11{\cdot}78$ are both some 8 p.c. greater than the values calculated from the uncorrected data.

We may conclude therefore that the effect of fatigue is comparatively unimportant. In vigorous male subjects it reduces the apparent theoretical maximum work (calculated from the observations) by some 8 p.c., and alters the apparent "viscosity" coefficient k to about the same degree. The character of the curve is unchanged by fatigue, there is the same increase of work with duration of contraction, the same equation applies with equal precision to the observations. The 10 p.c. to 20 p.c. difference found by Hansen and Lindhard(3) between W_0 and the area of the "stress-strain" diagram is clearly due, as they claim, in part to fatigue: unless, however, their subjects were more liable to fatigue than ours (which seems unlikely), it would appear that other factors must be invoked to explain the remainder of the difference.

The relation between work and speed of shortening can be shown diagrammatically much better by plotting W against $1/t$ than (as we have done hitherto) against t. If we call $1/t$ the "speed" of shortening, the relation between work and speed should be a linear one, as Fig. 1 indeed shows it to be. The lower line of Fig. 1 is drawn through the observations given by Lupton: the upper line is drawn through the observations after correction for fatigue, as described above. The linear relation shown

in Fig. 1 is the simplest possible expression of the facts demonstrated by the inertia wheel: the work done decreases in a linear manner as

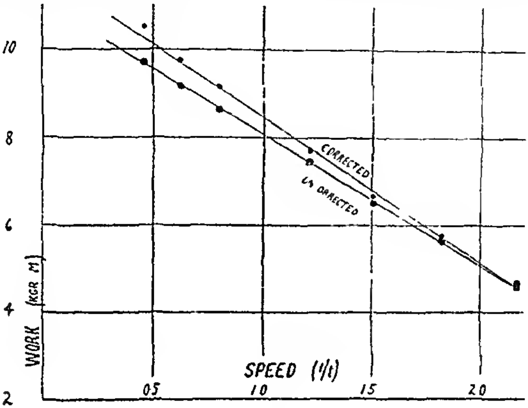

Fig 1

the speed of movement increases: and the small correction necessary to allow for fatigue obviously has no influence upon the accuracy with which this relation is obeyed.

Summary

The effect of fatigue in diminishing the work done in a prolonged maximal contraction of the flexor muscles of the elbow has been determined. Every previous 1 sec of maximal contraction diminishes the work by about 6 p.c. The relation between work and speed of shortening is not seriously influenced by fatigue. The work decreases in a linear manner as the speed of shortening is increased.

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increased or fell, and Fig. 1 shows all the results obtained with one animal.

Cane sugar 10 p.c. (26) + or + +. In one experiment there was a fall in resistance, which was clearly accidental.
Lactic acid trace to ·2 p.c. in 10 p.c. sugar (6) − + −, or + − +, or −.
·4 p.c. Na_2SO_4 in 10 p.c. sugar (4) + − +. or − +, or −, or +.
·1 p.c. NaCl in 10 p.c. sugar (2) + − +.
·1 p.c. indigocarmine in 10 p.c. sugar (2) − + −, or + − +.
3·4 p.c. NaI in normal saline (10) −.
5 p.c. Na_2SO_4 in normal saline (2) −.
5 p.c. NH_4Cl in normal saline (2) −, or + −.
·2 p.c. $NaHCO_3$ in normal saline (2) −.
Vagus stimulation (14) − except in two cases +.

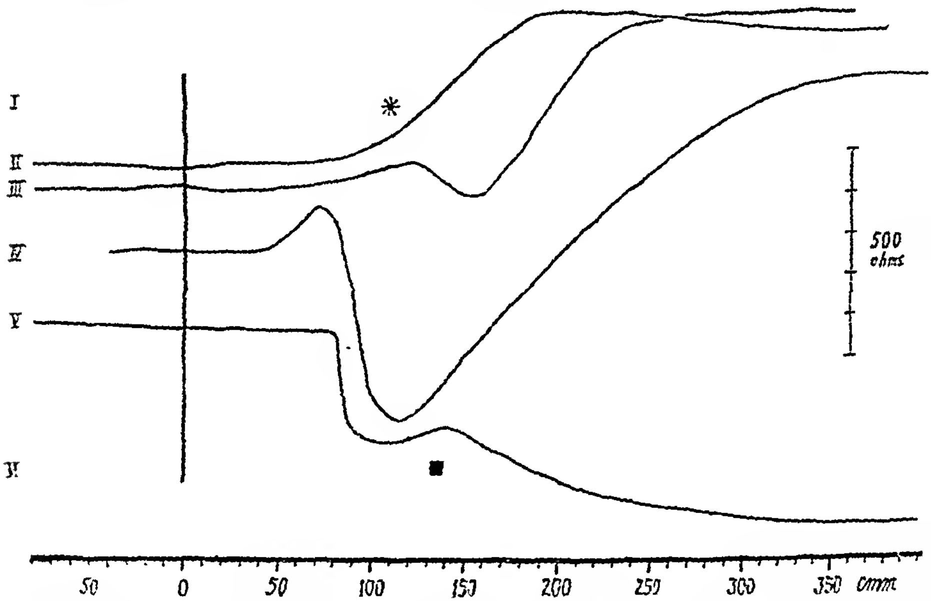

Fig. 1. Results on one rabbit. The lines show the changes in conductivity without regard to the initial absolute value. The time of flow of 10 c.mm. before injection is placed in brackets. I (21) urea, 1 c.c. 0·5 p.c. II (31) cane sugar, 1 c.c. 10 p.c. III (11) NaCl 0·1 p.c. in 10 p.c. sugar. IV (26) Na_2SO_4 0·4 p.c. in 10 p.c. sugar. V (11) vagus stimulation for 81 secs. VI (10) indigocarmine, 0·1 p.c. Cannula volume to be deducted, 30 c.mm.

It will be noticed that while many injections produce only one definite change in the resistance some, especially those which contained an electrolyte, added to 10 p.c. sugar solution showed up to three sharp points of change. Naturally the changes produced passed off and the resistance of the urine tended to approach its previous value. This change was usually gradual and in the beginning of such a return has not been

further considered. In some cases, however, the alteration was sharp. The turning-point in these has been used in the subsequent considerations. Naturally not all injections produced results. On occasion, for example, the strong sodium iodide solution produced no change; an injection of 10 p.c. sugar could, however, be relied on to give a result.

Stimulation of the vagus was the most frequent disappointment, largely owing to the desirability of limiting stimulation to the interval between the fall of two drops. When the urine was flowing rapidly such a brief stimulation produced no effect. Many of the vagus experiments showed a subsequent return of the resistance to the previous level or a passage beyond it. The quantity of urine expelled after the stimulation had ceased before the original level was reached has been taken account of in the subsequent considerations. In some cases the effect of the vagus was of a more permanent nature. Whether the influence of vagus stimulation is direct or merely a vascular effect does not affect the use to which it is put in these experiments; it may, however, be mentioned that a result was never obtained when the stimulus was too weak to stop the heart.

An inspection of all the observations showed that the volumes expelled before a change appeared tended, as in the previous experiments, to fall into two groups—small volumes and relatively large volumes—but there are many intermediate values. The results obtained cannot, however, be compared with one another unless allowance is made for the difference in size of the animals and still more for the difference in the state of distension of the kidney tubules and passages. The actual results obtained were reduced in simple proportion to conform with a body weight of 2 kilos.—the extremes being 1·5 and 2·5 kilos. The volumes so obtained were plotted against the corresponding rates of flow before the injection was given. This rate of flow must be in some close relation to the state of distension at the moment of injection, as the greater the rate of flow the greater the pressure causing it and consequently the greater the dilation of the tubules and ducts.

The results fall into order as seen in Fig. 2. In this all observations, including those of Part I, have been used. It will be noticed that with increasing rates of flow the volumes tend to become larger and the differences between extreme observations greater.

Along the lower edge of the figure there is an accumulation of points, indicated by the lowest line, which point to its correspondence with a definite level in the kidney passages. Characteristic of this line is further the fact that on, or near it, fall the initial changes of all vagus stimulations. It was consequently desirable to find as far as possible what is

the relation of this level to the volume of the ureter and pelvis of the kidney. This was determined in the following way. The usual preparation

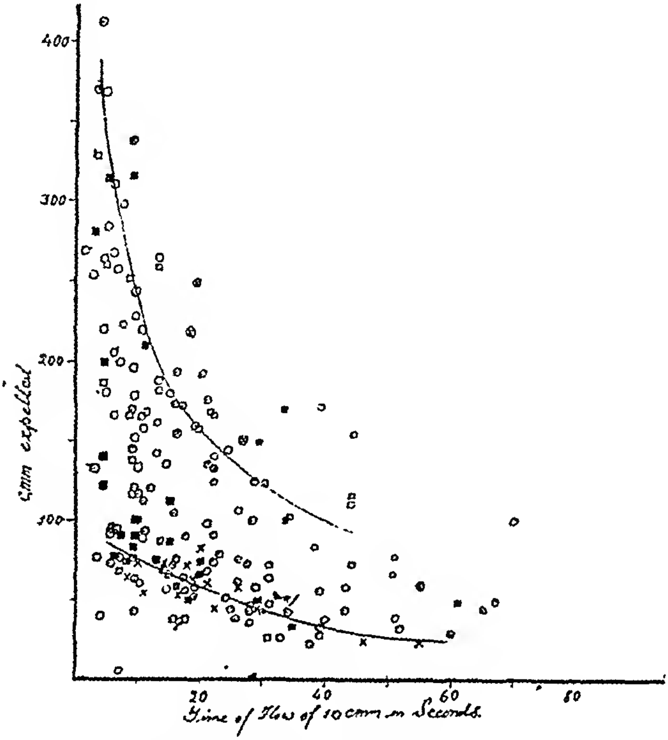

Fig. 2. The crosses indicate the beginning, and crosses in circles the ends of the conductivity changes with stimulation of the vagus. The black squares show the results with indigo-carmine, the white with iodide. The circles indicate all other changes.

having been made, indigocarmine was injected. A period was allowed to elapse until the urine was approximately constant in colour, a known number of drops were collected, the rate of flow taken, and the animal killed by an intravascular injection of chloroform. The ureter was clamped off and the kidney and ureter removed from the body. The kidney was now sliced away tangentially until the pelvis was just opened. The contents of the ureter were then washed out from the cannula with saline through this opening until the washings were free from colour. The collected washings were compared in a colorimeter with the previously collected drops to which, however, it was usually necessary to add a trace of blood. From the comparison, the volume of the drops being known, the volume of the ureter was calculated. While this volume does not include the volume of the kidney ducts which must constitute a further small addition to the dead space it does include the urine in the recesses of the pelvis which does not form a part of the

dead space in question. If the ureter volumes obtained in these experiments (Table I) be compared with Fig. 2 they will be seen to fall around the lower line. Consequently changes occurring at this level might be attributed to the lowest active region of the kidney tubules.

TABLE I. All values are reduced to a body weight of two kilos.

Wt. (kilo.)	Rate of flow before injection secs. per 10 c.mm.	Qt. expelled before colour appears (c.mm.)	Rate before death secs. per 10 c.mm.	Ureter vol. c.mm.
1·7	3·1	280	3·5	132
2·1	4·5	121	4	72
2·2	4·7	260	4	60
1·7	6·4	67	7·5	35
1·7	27	70	12	89
1·7	8·5	188	13	90
2	8·2	240	16	98
1·7	—	—	17	113
1·7	20	75	20	89
2·5	9·6	83	26	34
			Mean	81

It is of course clear that these ureter volumes do not reach to the volume to which the ureter can be distended; after obstruction to the outflow much larger volumes can be obtained. We have collected almost 2 c.c. of urine on cutting a completely obstructed ureter after death. Cases of obstructed outflow would reveal themselves on the graph as extravagantly large volumes at slow rates of flow. In one other determination of the volume of the ureter the rate fell in the course of 10 minutes from 7 secs. to 80 secs. per 10 c.mm. The volume of ureter was then found to be 280 c.mm.

If the experiments in the table be referred to it will be noticed that in some the volume expelled after the injection of the indigocarmine before the blue colour appears is of the same order as the volume of the ureter and pelvis while in others it is distinctly larger. Similarly, in Fig. 3, the upper limit of the observations form a definite group which it is natural to identify with the upper end of the active tubule as we identify the first with the lower end. Scattered between the two lie a number of points which may well be outlying members of the other two groups. We are, however, for the following reason, inclined to the belief that they correspond in part at least to another group. As already mentioned many conductivity experiments show three points of change in the resistance. If these alone be plotted in the same manner as in Fig. 2 it will be seen that while the extreme observations correspond in general to the groups just mentioned the middle ones seem to form an independent group (Fig. 3).

Before identifying these three groups with definite anatomical regions we must consider what other explanations of the results might be offered. Assuming for the moment secretion in the tubule, the time taken before a substance just arrived in the kidney blood stream would reach the

cannula would depend on the time taken in passing through the tubule wall added to the time taken to expel the urine already in the passages

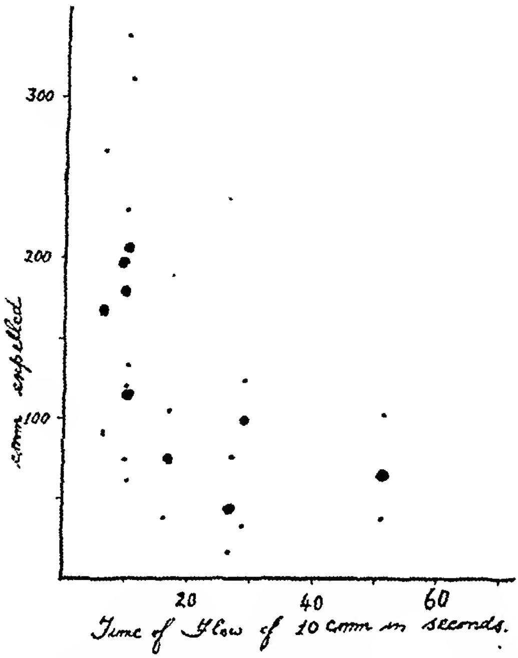

Fig. 3. Three changes in the conductivity after each injection. The large dot shows the middle change in each case.

below. If there be any noticeable delay in secretion, unchanged urine would continue to pass down the tubule and consequently the volume expelled before the change would be larger than if the secretion occupied a merely negligible time. That there is not any invariably appreciable delay is shown by the approximate correspondence of the volume of the ureter at various rates of flow with the lower line in Fig. 2. Delayed appearance of an injected substance is not, it is true, without a parallel; in chronic interstitial nephritis a delay in the appearance of injected dyes is described though even here pathological alteration in the volume of the ureter does not seem to have been taken into account (2). If, however, we are to explain any points in the graphs falling above the line corresponding to the anatomical volume of the ureter as delay in the transmission of a change in the blood through the active cells of the tubules it is not easy to see why they should accumulate along a particular line in our rate-volume graph. If instead of considering secretion one considers the activity of the tubule to be absorption the reasoning is obviously not essentially different.

The upper line indicates the latest change or series of changes we have

found. We have seen that the identification of them with delayed activity in the tubules is improbable and believe that this line indicates alterations in the uppermost portion of the tubule. We must then consider what arguments can, so far as we are aware, be directed against this view. It might be held that the volume of fluid in the uriniferous tubules is so minute that a separation from the point of view of volume between the upper and lower end is impossible. Such an argument would mean that observations grouped along the lower line of Fig. 2 may be due to glomerular activity, or activity throughout the tubule, or both, and that higher observations must be explained as due to delayed secretion or absorption. Such an argument would reject as misleading any evidence as to the volume of the tubules derived from histological sections, but in proof of the existence of a definite bore to the tubules we have the existence of tubular "casts" and also the fact that the tubules must have an appreciable bore so as not to require impossible pressures at the glomerulus to drive the urine along the tubule.

Admitting that the tubules have an appreciable volume it does not follow, however, that a change occurring in the glomerular fluid must expel their exact contents before it in order to emerge into the ducts. A delay in passing through the glomerulus is scarcely conceivable and the fluid in the capsule of Bowman will force the contents of the tubules before it, but these contents will be diminished by absorption or increased by secretion as long as they lie in the active tubule, and the volumetric difference between the extreme ends of the tubules so far as they can be measured in the emerging urine will resemble the anatomical content of the tubules only when secretion occurs in very concentrated solution. If absorption occurs to the extent demanded by the current absorption theories the volume difference reaching observation in our experiments would not be reduced to the same degree as only the urine which had to travel through the entire tubule would presumably be exposed to the full reduction and that in the lower end of the tubule would scarcely be affected. If we reject, as irreconcilable with the regularity of Fig. 3, the explanation of points along the upper line as due to delayed secretion or absorption we must hold them to indicate the volume of the uriniferous tubule less a quantity due to absorption or increased by secretion. Taking the number of tubules at 150,000 (3) rather than the very unusual figure previously obtained on a single kidney (1), and assuming all to be active, the difference between the two lines on Fig. 3 would be comparable to the volume of tubules of diameter of from about 4μ to 10μ. Histological sections would indicate a rather higher value.

We believe then that the first line corresponds to the lower end of the

uriniferous tubule and that the difference between the upper and lower line is related to the total volume of the uriniferous tubule. It is tempting to believe that the third or intermediate group (Fig. 3) is connected with the loop of Henle and the adjoining ends of the two convoluted tubules.

Wishing to find if the lines drawn through the chief groups of the observations corresponded to any mathematical relation, and graphing the volumes against the square root of the quantity of urine flowing in unit time it was found that the two upper groups of observations fell around straight lines drawn through the origin of the curve. Uncertainty, however, as to whether this relation is the most satisfactory does not make it work while considering deductions which might be made from it.

If the interpretation of the results which we have advanced be accepted, they permit the approximate mapping and in terms of volume and identification with anatomical structures of the active regions of the kidney.

It is at once clear that the vagus experiments could be explained equally easily by either of the two prevailing types of theory. The changes of conductivity after injections allow of but little more discrimination: the increase in resistance in a particular animal in the urine coming from the distal convoluted tubules after injecting a sugar solution would be most easily explained by the cutting off of the supply of electrolytes for secretion, but there is no direct argument against the suggestion that the absence of electrolytes around the tubule stimulated in some way an increased absorption of electrolytes. In cases like dilute Na_2SO_4 in 10 p.c. sugar where a decided fall in resistance of tubular urine occurred it is difficult not to explain the fall as due to a secretion of the sulphate. But especially in the absence of chemical analysis this is merely a probability. We consequently use the conductivity experiments only to map out the active region of the kidney and draw no further conclusion from them.

The case of substances previously absent from the urine and injected into the blood stream is different. If after the injection of indigocarmine we find indigocarmine in the urine which at the moment of injection occupied the distal convoluted tubule we must conclude that indigocarmine has been excreted by that region. The graphs show the occurrence of examples of both iodide excretion and indigocarmine excretion in all groups, and, so far as the interpretation presented convinces, there can be no doubt about their excretion in many cases by the convoluted tubules. Whether in those cases where excretion occurs in the convoluted tubules there is also excretion in the glomerulus cannot be decided by the experiments at our disposal.

If we consider finally the observations made on uric acid and urea, we find that in 10 out of 11 experiments uric acid appears to come from the convoluted tubule, while the two experiments with urea also

point to excretion by the tubule wall. As these substances are present in the previously passed urine it is possible to explain these results by an increased absorption resulting from the injection; but when we find. for example, an injection of indigocarmine showing itself in a urine which was flowing at the rate of 10 c.mm. in 15 secs. after the expulsion of 86 c.mm. and in the same experiment an injection of urea causing an increased concentrate of urea in urine flowing at approximately the same rate after the expulsion of 65 c.mm. it seems hard to refuse an explanation for the second result which alone accounts for the first.

The experiments then give direct support to the theory of the secretory activity of the tubule with the qualification that in some animals, presumably owing to an alteration in conditions, the glomerular excretion is alone demonstrable.

In conclusion we wish to thank Prof. J. J. Nolan for his advice on certain physical points and Prof. T. H. Milroy for helpful criticism.

Summary.

In continuation of previous experiments observations wcro made on the volume of urine expelled after an intravascular injection before a change in the urine occurred.

The main observations were made on the alteration of the electrical conductivity of the urine after intra-arterial injections of various solutions and on stimulation of the vagus.

Further experiments were made on the volume of urine expelled before injected indigocarmine or iodide or urea could be detected.

When these volumes are plotted against the rate of flow of the urine prevailing at the moment of injection the figures fall into groups of which an upper and lower are distinct and an intermediate one probable.

Volume determinations of the ureter and pelvis permit the identification of the lower group with the distal convoluted tubule and the upper group with the glomerulus.

The positions of the indigo and iodide observations on the graph point to the excretion point of these substances varying in different animals, indigocarmine being usually excreted by the tubule.

Observations on urea and uric acid point to these substances being usually excreted by the tubule.

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decreased under the effect of insulin. We obtained some evidence that an active process was concerned, as the following experiment will show. In a special experiment (Table I) the O_2-tension was controlled so that it reached 58·3 mm., *i.e.* about 20 mm. Hg above the normal—about 38 mm. for this animal—at the beginning of the insulin experiment.

TABLE I. Rabbit.

Time (*mins.*)	Partial pressure under skin mm. Hg CO_2	O_2	Blood sugar mg. %
0	38·6	58·3	·110
25 (insulin injected)	40·0	56·9	—
75	44·7	54·5	—
105	48·1	53·0	·055
155	50·4	50·4	—
162	—	—	·028
175 (convulsions)	—	—	—
195	61·9	48·1	—
235 (glucose injected)	—	—	—
266	—	—	·060
285	41·1	46·4	—
345	36·0	48·5	—
366	—	—	·112
405	34·0	52·0	—
18 hours later	37·4	39·0	—
66 ,,	39·5	37·4	—

Of course the O_2-tension fell slowly towards normal, but before it had fallen so far as this the blood sugar had fallen to its lowest point under the influence of insulin and following convulsions and subcutaneous injection of glucose was returning to normal again. Shortly afterwards the O_2-tension, which was 46·4 mm. Hg, that is, well above the normal level, increased again and remained on the upward trend for at least two hours. Next day the O_2-tension had fallen to normal, 39 mm. Hg. Macleod (4) points out that the respiratory quotient often rises decidedly in rabbits. This agrees with the rise in CO_2-tension and the fall in O_2-tension obtained by us.

From the above experiments it seemed clear that there was some relation between the O_2-tension and the blood sugar.

Air injected into the abdominal cavity. Our next experiments were designed to determine whether the effect was present throughout the body; for this air was injected into the abdominal cavity. The technique was similar to that for the injection of air under the skin; much finer hypodermic needles were used and smaller quantities of air were injected. In all experiments, five in number, no definite effects were observed.

Fig. 2 gives details of a typical experiment. The blood sugar in a rabbit fell, under the influence of insulin, from ·108 mg. p.c. to ·040 mg. p.c.;

the CO_2-tension in air in the abdominal cavity varied between 48 and 50 mm Hg and the O_2 tension between 29 and 32 mm Similar slight

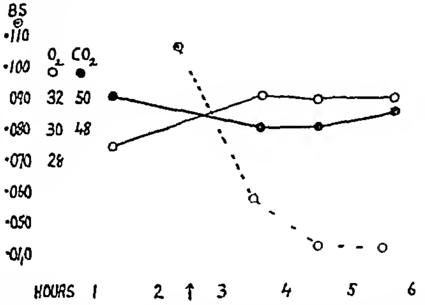

Fig 2 As Fig 1, but O_2 and CO_2 = tensions in air in abdominal cavity Rabbit, 2 kgm

variations of no definite character were obtained in the other four experiments We, therefore, concluded that the effects we had previously observed were not general but were localised near the skin and the muscles

The effect of vaso constrictor substances The effects of subcutaneous

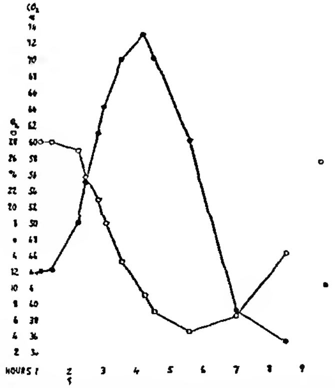

Fig 3 Injection of 4 c c "infundin" at arrow O_2 and CO_2 = tensions in mm Hg in air under skin Same rabbit as that of Figs 1 and 4 The observations on the extreme right were made 24 hours after the beginning of the experiment.

when the pH was about 7·4 dilatation was the most frequent result and the muscle often exhibited very marked twitching. Controls with the same preparations using a small drop of "Infundin" or adrenalin chloride invariably exhibited marked constriction. In other experiments, 20 in number, subcutaneous injection of insulin had no effect on the blood-vessels of the muscles even when enormous doses were given; small doses of "Infundin" or of adrenalin chloride administered in the same way produced marked constriction in these vessels. We concluded, therefore, that the effects of insulin on the gas tensions in air between the skin and the muscles were not due to constriction of blood vessels.

Summary.

After subcutaneous injection of insulin in normal rabbits the curve for O_2-tension in air between the skin and the muscles follows the blood sugar curve; the effect appears to be localised and is not due to constriction of blood vessels. Insulin also causes an increase of CO_2-tension in the same region, which lasts as a rule only a couple of hours.

We are much indebted to Dr H. H. Dale and to Dr Leonard Hill for their kindly interest, criticism and advice.

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STUDIES IN MUSCLE ACTIVITY. II. The influence of speed on the mechanical efficiency. By E. P. CATHCART, D. T. RICHARDSON AND W. CAMPBELL.

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THE question of the relation between the speed at which work is done and the mechanical efficiency with which it is carried out is of importance in determining the relative metabolic cost to an operative of working at different speeds. Observations on the question are not numerous and the majority of the earlier ones are complicated by the fact that with the rise in speed there was also a rise in the external work done. The highest efficiency found, for example, by Laulanié(1), was 23·3 p.c. with a load of 4 kilos and a speed of 0·61 metre a second. With an 8 kilo load and a rate of 0·37 metre per second the efficiency fell to 20·7 p.c. and with a 12 kilo load and a rate of 0·24 metre a second it fell further to 17·0 p.c. Laulanié has an interesting discussion on economic and mechanical optima or efficiencies.

Benedict and Cathcart(2) also carried out a series of determinations with varying speed, in the majority of which the load was not kept constant. They found very good evidence that the mechanical efficiency diminished as the speed of performance (on a bicycle ergometer) increased. They also carried out a more limited set of experiments where the influence of speed alone on efficiency was examined, the amount of external muscular work done remaining constant. Here it was shown very definitely that a marked diminution in the efficiency took place with increase of speed. Thus when the heat equivalent of the muscular work done per minute was approximately 1·95 cals. per minute, the average net efficiency with a speed of 90 revolutions per minute was 22·6 p.c. and with a speed of 124 revolutions only 15·7 p.c.; with a heat equivalent of 1·80 cals. per minute at 80 revolutions there was an efficiency of 22·1 p.c. and at 105 revolutions 17·7 p.c. The highest efficiency in their subject was found to be at the rate of 70 to 80 revolutions per minute.

A. V. Hill(3), in a very interesting series of observations, which he made on the flexor muscles of the arm, corroborated in a very definite way the findings of Benedict and Cathcart. He noted that the

efficiency passed through a definite maximum value as the duration of the contraction was increased. He found in practice that the optimum time of contraction was about 1 second. He also found that by decreasing comparatively slightly the time occupied in a muscular movement a serious loss of efficiency might be caused and that a comparatively large increase in time might cause only a small loss in efficiency. We are inclined to accept the explanation of Hill that the more or less general agreement of practically all observers that the mechanical efficiency of man lies between from 20 and 25 p.c. is due to the fact that the maximum is "blunt," *i.e.* that over a comparatively wide range of speeds the efficiency remains approximately constant.

More recently Lupton(4) working in Hill's laboratory has determined the optimum rates for two other types of work. He found for stair climbing that the optimum duration for a single contraction was about 1·3 seconds with an efficiency of about 24·4 p.c. He also determined the rate for the simultaneous contraction of the flexors of both arms and found a mean efficiency of 26·7 p.c. and a mean value for the optimum duration of a single contraction of 1·36 seconds.

It is interesting to note in this connection that the optimum or more efficient rates for two other types of movement have also been determined. Thus Cathcart, Richardson and Campbell(5) found in their latest experiments on marching that, in common with several previous workers, the optimum rate was about 80 metres per minute or, if the pace be considered, about 100 paces per minute. Stevenson and Brown(6) found that the optimum rate for shovelling was 17 to 19 throws per minute and for picking 25 to 30 strokes per minute.

The present set of experiments were carried out on the variable ergometer recently described(7), in which a very wide range of speeds and loads could be obtained. Two thoroughly experienced subjects, R. and C., in perfect training, who had served for similar determinations of respiratory metabolism for well over a year and who were, therefore, thoroughly acquainted with the technique thus obviating one of the main sources of error in this type of experiment, were used. R. was 174·5 cm. high and weighed 70·3 kilos: C. was 168 cm. high and weighed 67·4 kilos. Arm movements were alone used, the subjects taking every care to confine the movement so far as possible to the muscles of the arm. Each experiment was of an hour's duration and the rate of work was given by the beat of a metronome.

The amount of external work performed in the course of an hour's experiment amounted in each instance to approximately 12,000 kilo-

gram metres, but the rate at which the work was done as is shown in the following table (Table I) varied widely The whole series was carried out at the full stroke of the levers

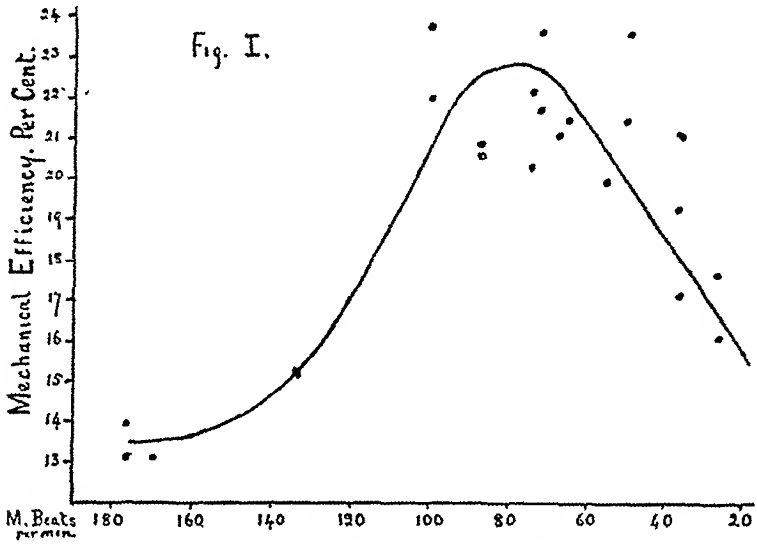
TABLE I Work done constant Speed, gear and load variable Subjects R and C

Gear	Load in kilos	Metronome rate per min		Work done in kgm per hour		Cals per sq m per min		Grm cals per kgm		Oxygen in c c per kgm		Mechanical net efficiency %	
		R	C	R	C	R	C	R	C	R	C	R	C
Low	1 5	177	177	12,058	11,538	1 761	1 947	16 87	17 91	3 21	3 67	13 89	13 09
	2	133	133	12,189	11,892	1 686	1 715	15 35	15 31	3 05	3 09	15 26	15 31
	3	88	88	12,060	11,754	1 240	1 248	11 40	11 28	2 27	2 26	20 55	20 70
	4	66	68	11 844	12,150	1 153	1 273	10 67	11 13	2 20	2 24	21 46	21 05
Mid	1 5	100	100	12 004	12 006	1 153	1 108	10 64	9 80	2 15	2 01	22 02	23 93
	2	75	75	11,088	11 811	1 253	1 177	11 60	10 59	2 32	2 14	20 20	22 16
	3	50	50	12,294	12,245	1 096	1 258	9 90	10 91	2 00	2 18	23 69	21 47
	4	37	37	11,916	11,952	1 241	1 539	11 09	13 69	2 24	2 75	21 14	17 14
High	1 5	72	72	11,932	11,752	1 162	1 095	10 79	9 90	2 15	2 02	21 72	23 71
	2	55	56	12,075	12,285	1 203	1 360	11 07	11 56	2 20	2 36	21 18	19 94
	3	36	37	11,975	12,033	1 198	1 377	11 10	12 16	2 23	2 47	21 13	19 32
	4	27	27	11 988	11,826	1 450	1 648	13 27	14 63	2 71	2 99	17 73	16 03

As regards the work done, in spite of the variation in the rate at which it was carried out, it will be noted that it is wonderfully uniform The average per hour for R for the whole series was 12,029 kgm , for the low gear it was 12,038, middle gear 12,051, and for the high gear 11,998 kgm per hour In the case of C the general average was 11,937 for the low gear 11,834, middle gear 12,004, and for the high gear 11,974 This gives a general average of 11,983 for both series together The slight variations in work done would seem to bear no relation either to the increase of load or to the diminution in speed

When the relation of the speed of performance to the metabolic cost is investigated it is very obvious both from the study of the cost in grm cals and the oxygen consumption per kilogram-metre of external work performed that the cost is heaviest at the higher rates of speed, that there is a reduction in the cost as moderate rates of speed are reached and that finally there is a rise when the rate becomes slower, *i e* as the static component during the performance of the work becomes more pronounced This is in good agreement with the previous observations of Cathcart, Bedale and McCallum (8) This variation in cost of course means that the mechanical efficiency with which the work is done must vary This is seen very clearly in the graph (Fig 1) which is built up from the total observations on both subjects The general result is that in these experiments with arm movements of a very simple order the maximal efficiency of practically 23 p c is reached with a rate of about

80 to and fro movements per minute. Subjectively the subjects found this a very easy rate to work at and it agrees with observations which were



made on another highly skilled subject of good physique and of similar build to subject C. who was allowed in a series of experiments to select his own rate of movement.

When the individual subjects' records are examined it is found that R. apparently reached his maximum efficiency at a rate of 50 beats with a load of 3 kilos on the middle gear. The efficiency figure nearest to it is also on the middle gear with the 1·5 kilo load and at a rate of 100 beats per minute. If this in reality be the highest value then it agrees with the observations on C. who was found to reach his maximum efficiency with this load and rate. As the oxygen consumption value for the 3 kilo load at 50 beats per minute with the middle gear seemed to be rather low, and as the error, if any, did not lie in the analyses of the samples which were made in duplicate by two observers, this particular experiment was repeated in another series on R. The mechanical efficiency in this series was found to be in astonishing agreement with the first. In place of 23·69 the repeat determination gave 23·7 p.c., the net grm. cals. per kgm. being 9·88 in place of 9·90 and the oxygen consumption per kgm. 1·95 in place of 2·00 c.c.

In this second series of experiments all three gears were used with constant load but at variable speed. This means, of course, that the amount of external work done increased steadily with the increased speed of performance. As the following table (Table II) clearly shows there is

a very definite variation in the mechanical efficiency with which the work was done.

TABLE II. Speed and gear variable. Brake load constant (3 kilos) Subject R.

Speed															
Metronome beats per min.	Actual (wheel) revs. per min			Work done kgm per min.			Net grm. cals per kgm.			Oxygen in c c per kgm			Net efficiency %		
	Low	Mid	High	Low	Mid	High	Low	Mid	High	Low	Mid	High	Low	Mid	High
50	25 6	45 2	61·7	115 1	203 6	291·1	12 31	9 88	10 59	2 51	1 95	1 91	19 1	23 7	22 1
60	30 5	53 9	76·0	137 1	242 5	342 0	10 67	9 98	10 83	2 15	1 99	1 95	21 9	23 5	21 7
70	35 4	62·0	87·4	159·4	279 1	393 3	10 13	9 74	10 82	2 03	1 93	2 01	23 2	24 1	21 7
80	39 9	71 8	99 5	179 6	322 9	447·8	10 06	9 50	11·06	2 00	1 86	2 07	23 3	24 7	21 2
90	46 3	83 3	111 9	208 5	374 8	503 6	10 66	10 62	10 50	2 12	2 09	2 03	22 0	22 1	22 3
100	50 5	89 5	126 9	227·1	402 8	571·0	10 18	11 57	11 53	2 04	2 25	2 26	23 0	20 3	20 3
110	55 1	98 4	137 4	248 0	442 9	618·1	10 81	11 38	11 77	2 15	2 22	2 31	23·4	20 6	19 9
120	60 6	107 5	—	272 7	483 7	—	11 60	12 02	—	2 31	2 35	—	20 2	19 5	—
130	65 9	116 9	—	296 4	526 2	—	11 74	12 09	—	2 36	2 37	—	20 0	19 1	—
140	71·1	123 5	—	319 8	555 6	—	12 28	13 98	—	2 46	2 74	—	19 1	16 8	—
150	75 5	—	—	339 8	—	—	12 65	—	—	2 52	—	—	18 5	—	—
160	80 5	—	—	362 0	—	—	13 79	—	—	2 74	—	—	17 0	—	—

Gear ratios: Low, 1:1; Mid, 1:1·7; High, 1 2 4

If the low gear experiments be considered first it will be noted that the efficiency with which the work is performed rapidly rises from 19·1 p.c. to a maximum of just over 23 p.c. when (with the exception of a single observation) a plateau would seem to be reached which covers the metronome rates from 70 to 110 beats per minute or in actual revolutions of the flywheel from 35·4 to 55 revs. per minute. In terms of external work done it means that there is little or no variation in efficiency between the production of 159·4 and 248 kgm. per minute. This plateau is followed by a rapid decline, the efficiency of production falling to 17 p.c. with a revolution rate of 80·5 revs. per minute (met. rate 160) and the production of 362 kgm. of work per minute.

In the case of the middle gear the maximum efficiency is reached with a metronome rate of 80 beats, *i.e.* a wheel revolution rate of 71·8 with the production of 322·9 kgm. per minute. There is, however, but little diminution in the mechanical efficiency with which the work is done with the slower rates tested, but the efficiency rapidly declines with the rise in the rate, the lowest efficiency, 16·8 p.c., being reached with 130 beats per minute.

In the case of the high gear it would seem that the maximum efficiency is reached with the slowest rate of working tested with the production of 291 kgm. per minute, although there is an equally high efficiency obtained at 90 beats per minute. Speaking generally, one might say that there is more or less of a plateau ranging from 50 to 90 beats per minute with a subsequent definite decline.

It may be noted that subjectively the limit of sustained effort was not reached on the low gear at 160 beats per minute and the production of 362 kgm. of work, whereas with the mid-gear the limit *was* reached with 130 beats and the production of 526 kgm. per minute, and on the high gear the subjective limit was at 90 beats with 504 kgm. per minute. The subject was quite positive about these limits. If both the efficiency as determined and the subjective symptoms be taken into account the inference may be drawn that with arm movements the sustained production of between 500 and 600 kilogram-metres of work per minute is about the maximum which can be done by the average subject. A number of experiments have been carried out for another purpose on still another subject very muscular and highly trained. He was tested on many occasions over one hour's continuous work and frequently managed, with comparative ease, the production of 600 kgm. per minute, and on two occasions he managed with difficulty to do just over 40,000 kgm. in the hour or approximately 670 kgm. per minute. This subject found that his optimum rate of work for long periods was about 400 kgm. per minute. This meant, however, very steady and uninterrupted work.

Incidentally in connection with the first series of experiments on R. and C. it is interesting to note the influence of muscular work on the respiratory quotient. In each experiment in addition to the pre-work lying value (the subject lay at complete rest before this determination for 35 to 40 minutes) three determinations of the metabolism were made during the course of the hour's work. As the following table (Table III), where the average R.Q.'s of all the experiments are grouped under their respective gears, shows there is first a definite rise in the R.Q. after work

TABLE III. Variations in Respiratory Quotient with work. Subjects R. and C. Work done, constant.

			Work determinations					
	Basal		(1) 20 minutes after start		(2) 38 minutes after start		(3) 57 minutes after start	
Gear	R.	C.	R.	C.	R.	C.	R.	C.
High	·804	·835	·893	·901	·896	·886	·886	·866
Mid	·818	·847	·900	·911	·884	·900	·876	·882
Low	·831	·820	·949	·917	·921	·906	·918	·887
Mean	·826		·912		·898		·885	

commences and this is followed by a lower R.Q. in the second determination and a still lower R.Q. during the third determination. It is highly improbable that the definite rise in the first determination is due to pumping out of carbon dioxide as this examination was never made until

20 minutes after the start of the working period when it is almost certain respiratory equilibrium had been regained In any case both the second and third determinations were also higher than the pre work determination Benedict and Cathcart(2), in the course of their experiments observed a similar tendency in the behaviour of the R Q 's in their highly trained professional subject during the work periods It would appear to be fairly conclusive evidence in favour of the selective combustion of carbohydrate induced by muscle activity

Conclusions.

1 When the amount of work done in unit time remained constant but the rate of performance varied it was found that the mechanical efficiency with which the work was done was low with very fast and very slow rates and high with medium rates

2 The highest efficiency obtained for arm movements under the above conditions was about 23 p c

3 The optimum rate of working would seem to be about 80 beats per minute

4 When the amount of work done in unit time was allowed to vary, a maximum efficiency of 24 7 p c was obtained at the same rate (80 beats per minute) of working

5. The sustained production by arm movements of between 500 and 600 kilogram-metres of work per minute would seem to be about the maximum output for the average man

6 The performance of muscle work brought about a rise in the respiratory quotient above the pre work resting value

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MITRAL INSUFFICIENCY. By D. T. BARRY.

(*From the Institut Marey, Boulogne-sur-Seine, and University College, Cork.*)

The question in what period or periods of the cardiac cycle regurgitation through a defective mitral valve may be set up experimentally is one which has exercised the minds of a limited field of workers in recent years. Some of those, as Wiggers and Feil (10), maintain that the back flow in an experimental lesion occurs chiefly if not entirely during the phase of ejection and for about 0–·08 of a second after the closure of the semi-lunar valves. Others, as Straub (8) and Schwartz (6), hold that it may be free in the isometric stage of systole, but in such event the early part of systole could not be isometric. The present writer has been for some two years past engaged in the investigation of mitral lesions in the dog, using the heart lung method of Starling which he believes to possess many advantages. A few only of the records of the experimental work are given in the present communication, the bulk of them will form the subject of a future paper. This communication deals chiefly with a case of heart affection in the human subject.

As the optical recording method employed in the investigation gave somewhat different curves for the apex beat from those to which we are accustomed with levers, a normal cardiogram taken by the former method with sensitive tambours is first presented in conjunction with the arterial and venous pulses (Fig. 1). The vibration frequency of the apparatus generally employed was about 150 per second for the apex beat and artery while for the venous curves it was less than 100. But the record is typical of the form got with various vibration frequencies. There are four elevations on the apex curve, numbered for convenience 1, 2, 3, 4. The first coincides with, or follows closely, the *a* wave of the venogram, the second, corresponding with the *c* wave, presents a notch at the top which apparently marks ventricular output. Succeeding this is a dip during ejection, followed by a third elevation on the top of which is a second notch about the point of closure of the semi-lunar valves. Then there is a dip and lastly a fourth or diastolic rise synchronous with the fall of the *v* wave of the venous record. What part of the cycle is represented by the second elevation of the apex graph at its inception? It varies

slightly in position in relation to the *c* wave in different records according to the phase of respiration, position of tambour, facility of transmission to chest wall, etc. but on the whole is fairly constant in coinciding with the c wave and therefore probably reveals the initial stage of ventricular systole in most normal tracings. No. 1 may undergo slight shift by transmission.

Fig 2 Fig. 1.

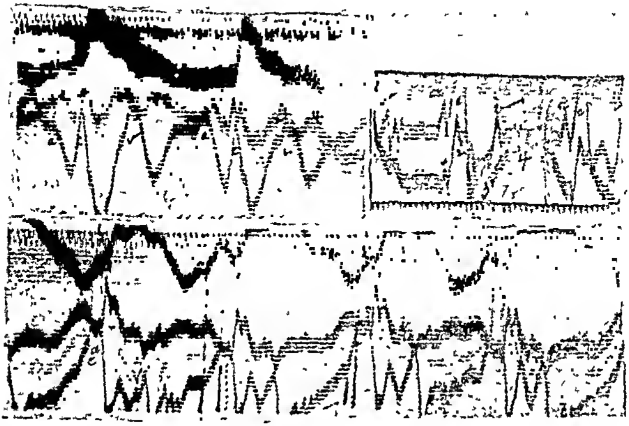


Fig. 3.

Fig. 1. Optical record of venous pulse (bottom), apex beat (middle) and carotid artery (normal). Time, 1/30 sec. in all figures

Fig. 2. Venous (upper) and apex curves from a case of mitral disease in man. Contrast with Fig. 3 The last cycle in this is added after cutting away a good piece of record to show some change of form in apex curve.

Fig. 3 Mitral lesion after exercise and eating. *Pulsus alternans* in arterial curve (top) *A* and *C* blended in venous curve (bottom). Large early *C* wave shows regurgitation in "isometric" systole. Double *V* wave, 1st part communicated.

A case of double mitral lesion in a youth of seventeen years, kindly sent to me for instrumental examination by Dr Cagney showed some features in the optical records which suggested the occurrence of back flow in the very earliest period of systole and presented some variations of records which are of much interest from another point of view. In the quiet resting position after a long period of inactivity we see on the veno-

gram (Fig. 2) a distinct *a* wave, a fairly large and sharp *c* and a compound *v* wave. The *c* of the venogram precedes No. 2 elevation of the apex curve by a short interval (0–·02 sec.) but there is a tiny separate elevation on this latter curve which corresponds to *c*. The main upstroke, No. 2, is still presphygmic, there being a delay in tension development and a delay in communication to the chest wall owing to a leak at *c*. Some evidence for this view will be given presently.

A graph from this case of mitral disease after exercise and the taking of a substantial meal (Fig. 3) reveals certain features which can be best explained by early regurgitation. The first thing to note is that *pulsus alternans* has supervened where previously the beats were uniform. As occurs in this phenomenon generally, the heart cycles are uniform in duration; the arterial curves are unequal, and the venogram shows alternation of a discordant type. The *a* and *c* waves are now blended in the venous pulse (bottom), the *c* wave being shifted to the left of the ventricular rise of the cardiogram by about 0–·06 sec. The *c* wave rises distinctly higher with the small arterial cycles than with the larger. This suggests a greater back flow in the smaller beats, and the leak would of itself account for a smaller volume being available for output in these. The origin of *pulsus alternans* is still hypothetical, but Gaskell's idea is generally accepted, namely, that it is due to suppression of activity in certain groups of muscle fibres in alternate cycles (Lewis (5)). Alternation in the auricle accompanying that in the ventricle refers ordinarily to variation of auricular systole, not of ventricular elements in auricle curves; but it is just this latter form of variation which constitutes the chief peculiarity of the present record, because it is discordant with the ventricular change. Awaiting further evidence to be presently given let us take it that early leak reduces the volume of blood available for output in alternate cycles and we may look upon this leak as a causative factor in *pulsus alternans*, but the question remains, why there should be greater back flow in alternate cycles. A glance at the carotid curve will show auricular systole or the *a* wave of the venous pulse on the upstroke, and this is larger on the larger cycles, from which it may be inferred that there is a greater output from the auricles in these. The cardiogram gives evidence of the same, and on the venous curve the thick part of the line running into *c* which represents the *a* wave is more sloped in the large beats and indicative of larger volume. The suggestion that fluctuation occurs primarily in the activity of the auricle, that of the ventricle being a passive effect of volume change. does not explain the condition. Mere change of force in the beat of the auricle would not

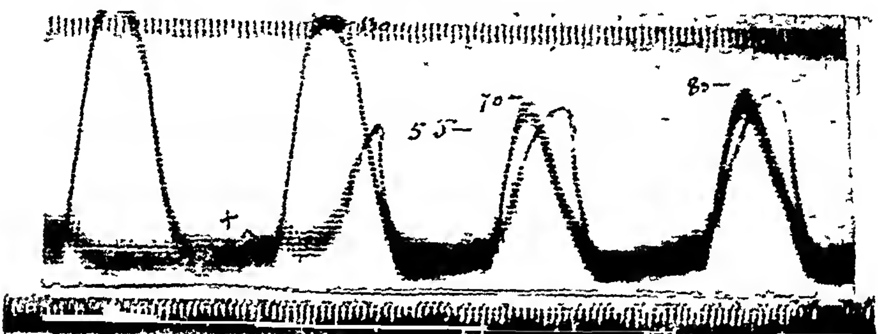
add appreciably to ventricular volume. It must seem anomalous that the greater back flow should accompany the weaker beat with the lesser input to ventricle, but the time and extent of closure of the auriculo-ventricular valves varies according to intraventricular pressure. as we shall see in a moment, and this variation can account for the phenomenon. This view is supported by certain curves obtained in experiment which are to be explained only by variation of valvular efficiency. To complete the analysis of this record it is to be noted in the venous curves that there is a double *v* wave or rather that the *v* is preceded by an extra elevation. This supplementary rise alternates in concord with the arteriogram and must be looked upon as a communicated impulse from the arterial side.

Wiggers and Feil (10) in their investigation of regurgitation phenomena relied upon a difference of slope of the ventricular curve of pressure as a necessary accompaniment of presphygmic back flow which must prolong the "isometric" period of systole. But the height and duration of the ventricular element of the venous curves must be taken as having considerable value in the same direction, and especially is this so when augmentation of this element coincides with the lower intraventricular pressure in the condition of alternating pulse. It may be said that the lower pressure is merely the *result* and in no way the *cause* of the greater leak which gives rise to the augmentation of the element, if this last point is conceded, but with uniformity of valvular defect in all cycles we should then have to ascribe increase of back flow to increased *initial* volume. which, considering the reduced output in these cycles, would be a greater anomaly than the causation of bigger leak by smaller volume. For the realisation of the latter alternative it is necessary to suppose that the aperture in the valve is bigger in the small cycles than in the large, and this will be shown to be not merely possible but very probable in this case. As long as the valvular defect remains uniform the greater leak must be with the greater force and volume in the ventricle, and so too for the output to the arteries. Another possible factor, namely fluctuations of resistance from auricular pressure changes, has to be mentioned, but there also an early leak from the ventricle must be granted to account for it. It may be that primary inherent fluctuation of auricular activity would determine a greater back flow in small than in large beats with no variation in size of the opening, but it must be remembered that the back flow is not synchronous with auricular systole during which the pressure differences would be marked. If the increase in size of the ventricular element of the venous curve were accompanied by a rise of ventricular

pressure it could be the result of an extra push against the closed valves. but occurring with reduced pressure it can only be due to regurgitation.

Increased slope of the intraventricular curve in the presphygmic period can be seen in regurgitant cycles of an alternating record from an experimental lesion (Fig. 9). And this slope does away with any idea

Fig. 8.



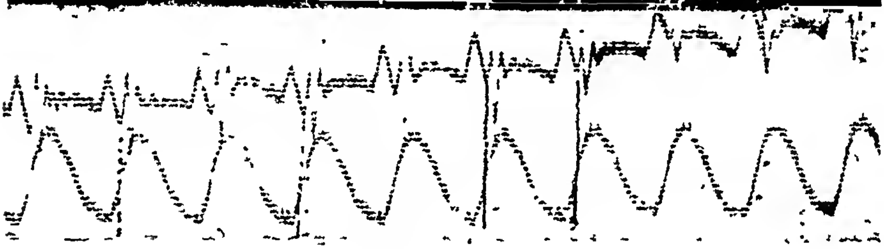


Fig. 4

Fig. 8 Successive beats 2, 3 and 4 show increasing volume of back flow. Lesion set up at X. The figures indicate ventricular pressures.

Fig 4 Increase of venous flow affects auricle curve (upper) but has little effect on ventricle. Note carefully the outline of ventricular upstroke. It shows different degrees of slope, the central thin part of the line marking the isometric phase.

that the larger *c* wave. or its equivalent deflection. may be due to a rapid rise of initial tension without early back flow. Such difference of slope may not be so marked, however. and the fraction of time lost in the ventricle can only be detected in simultaneous aortic curves. In some aortic curves in the present experiments delay in the opening of the valves could be detected when early regurgitation was supposed to exist (Fig. 5), and this with or without apparent change of slope in the ventricle line.

The drop in auricular pressure, the downstroke of the ventricular element of it, has been considered difficult to explain in early back flow (Wiggers and Feil), as the pressure should remain up through the

sphygmic period. But a sudden diminution of force of the regurgitant jet such as must be caused by the opening of the arterial side path should be sufficient to cause a quick fall.

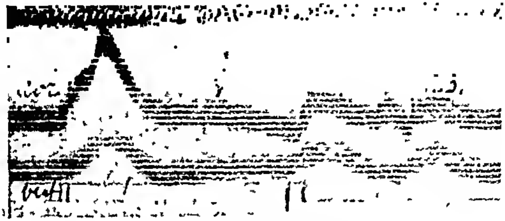


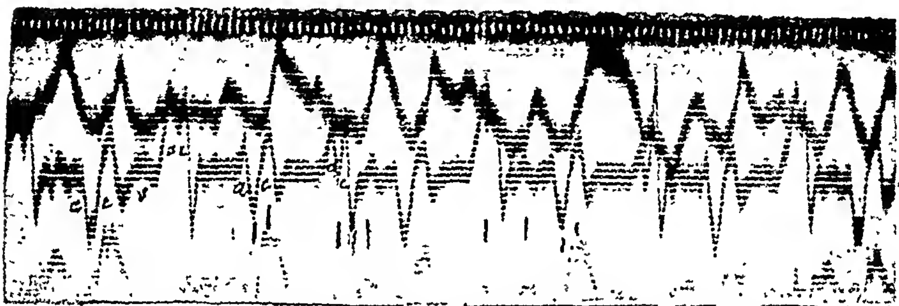
Fig. 5. Intraventricular pressure (lower) and aortic pressure curves Regurgitation at arrow.

Exercise and food did not always induce *pulsus alternans*, but sometimes gave rise to irregularity of rhythm, premature beats, etc. (Fig 6). Records of the heart sounds (Fig. 7) from different parts of the chest wall gave a well-marked second sound with some slight appearance of first sound in alternate beats.

Wiggers and Feil produced some very convincing records in support of their view and their interpretation of them is, I consider, quite sound In criticising their work I acknowledge much inspiration derived from it Other things, however, may happen than those which they disclosed. They could not provoke the same type or degree of change that one can provoke with the heart-lung method. The infusion of saline fluid for varying the venous flow is not to be compared with that of blood, which obviates changes of viscosity and composition and differences of temperature. The blood cools rapidly in the lungs even with well-warmed air for respiration and the temperature of the body does not always indicate that of the blood in the left side of the heart. Slight change of temperature may affect the heart considerably, and these are entirely excluded by artificial circulation. Gradation of aortic resistance can also be much more finely effected. Gradation of the amount of leak is an aspect to which I have devoted particular attention. Having first used a catheter for the production of the lesion as mentioned elsewhere (1) I discarded this for a tube and plunger such as described by Wiggers and Feil. This has been inserted not through the ventricle, as they did, but through the auricular appendix. The clamping of two rigid tubes in the ventricle,

for the manometer must also be clamped, should at least hamper the shortening of a band of muscle fibres stretched between them. The tube

Fig. 6.



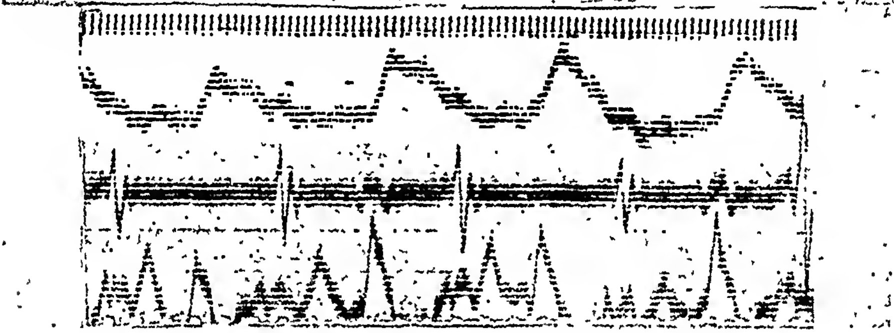


Fig. 7.

Fig. 6. Venous and arterial curves from a case of mitral disease after food and exercise. Irregular pulse—no alternation proper.

Fig. 7. Record of heart sounds (centre). First sound absent.

used had an elongated or slit-like opening which could be uncovered to any desired extent by partial or complete withdrawal of the plunger, and so cause large or small leak. The clotting of blood in natural conditions is also a disadvantage.

So far as these experiments have gone they indicate that leakage through the mitral valve can occur in any phase of ventricular systole from the earliest moment of increased tension, and that in certain conditions of auricular and ventricular pressures the tendency to early back flow increases with increase in size of the leak. The lower the auricle pressure, or the greater the difference between it and ventricular pressure the more easily does presphygmic regurgitation occur, but low ventricular volume *per se* favours it. Curves taken with a gradual increase of leak by slow withdrawal of the piston usually show in successive cycles not

only a graded increase in auricular pressure from back flow but a gradual shortening of ventricular time in causing it (Fig. 8). On the other hand with a fair sized leak when the difference of pressures in auricle and ventricle is not marked the time corresponds pretty well with that of Wiggers and Feil and is to some extent an indication of compensation taking place. Compensation is more difficult to establish with early than with late back flow. The gradual reduction of the time in the present case occurred with a flow of little more than 200 c.c. per minute, an auricular systolic pressure of about 6 cm. of water and an arterial pressure of 80 mm. of mercury. The intraventricular pressure fell from 100 mm. of mercury to 80 on setting up the full leak. The aortic pressure which corresponded exactly with the regurgitant cycles could not be determined but the fall was at first slight and the pulse pressure available must have been very low. These conditions may be considered exceptional but similar ones can occur naturally. In them compensation of a small leak may be easy, of a large one impossible. When the auricular pressure is high, regurgitation is slight in the early phase of systole with an artificial lesion, but with a large leak there is always indication of it at least for a few beats after the lesion is set up. It is not purely a question of difference between auricle and ventricle pressures; the size of the opening which remains patent and the degree of resistance offered are also factors in determining the time of leakage. The pressures were measured against a mercury manometer. Shifting of the beam could always be detected by closure of the bottom tap of the manometer and opening the top one, which at once gave zero pressure within it.

Of more interest in the present connection than the last mentioned experiment is the result of another in which *pulsus alternans* supervened. It resembles in some features the case of natural valvular disease already dealt with. This record (Fig. 9) raises again the question of what closes the auriculo-ventricular valves. Henderson and Johnson (4) reopened this in 1912. They considered the closure to be due to the cessation of the auricular jet as was suggested by Baumgarten in 1843 (2). Henderson and Johnson believed they effected closure in a model by this agency and argued that if it were not so, some regurgitation must occur in the very early phase of systole. Wiggers (9) is of an opposite opinion; he maintains that the final closure of the valves is due to the rise of intraventricular pressure but that it occurs so readily that regurgitation does not take place. If the valves were sealed only by the rise of intraventricular pressure the presphygmic period of contraction should not be considered altogether isometric, even if there were no

regurgitation, but Wiggers thinks it is not worth while dividing this phase into two. Straub(7) holds that auricular systole causes distinct increase in ventricular volume, and closure of the auriculo-ventricular valves gives rise to variation of ventricular volume curves according to the preponderance of push or pull upon them—push from the pressure developed and pull from the contraction of the papillary muscles. The pull is necessary for efficient closure to counteract the effect of the push and the question very naturally arises if the converse be true or not. Careful inspection of normal curves of auricle and ventricle taken by the optical method in the heart-lung shows three phases on the upstroke of intraventricular pressure (Fig. 9), a lower thick portion, the *upper* limit

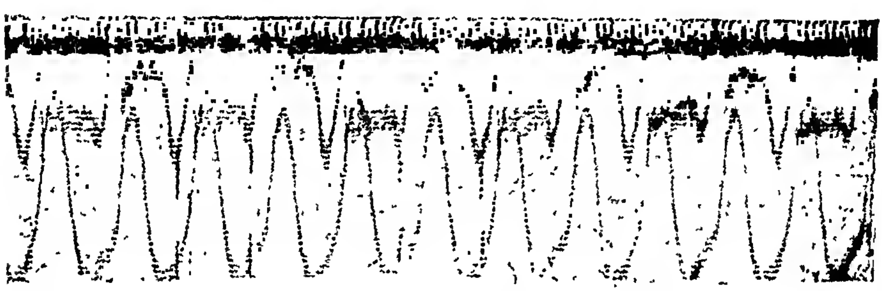


Fig. 9. *Pulsus alternans* with alternation of mitral beat in experimental lesion. Explained in text. The time record and the apices of the curves which cut it have been slightly retouched. Cycles ·25 sec. and ·32 sec.

of which corresponds with the beginning of the ventricular element of the auricle curve, a middle thin part more vertical, the upper limit of which corresponds with the tip of the ventricular element, and a third thick portion with increased slope. It seems clear that the first part represents a phase of systole which is not isometric, a part during which the *a, v* valves are being pushed up into position, the second represents isometric contraction with both valves sealed, and the third marks output. There is no evidence of early leak either with moderate inflow (beginning) or with large return (end of record).

On the view that the valves are only fully closed by the rise in pressure and accepting the opinion that it does not cause early back flow in healthy conditions, leakage can, however, be easily conceived as occurring with defective closure in the phase referred to above as isometric. Henderson(3), in contradistinction to Straub, thinks that the contraction of the auricle increases ventricular volume only to the extent of a few drops and Wiggers agrees, apparently, with this. Now there are certainly variations of auricular force and tidal flow in valvular defect which must have some effect on ventricular volume, variations which may occur naturally in

disease and which can be produced experimentally. The form of *pulsus alternans* set up in this way (Fig. 9) is best explained by the regurgitation tube having been inserted rather far so that its opening engaged in the auriculo-ventricular valves in alternate cycles. There is alternation of ventricular pressure with evidence of back flow in the lower beats, and this not only in the sphygmic period but in the early phase of systole as shown by the larger ventricular wave of the auricle curve. At the end of each of these regurgitant cycles there is a marked fall of auricular pressure and a big flow to the ventricle, a consequent higher ventricular pressure and a sealing of the leak in the next beat by more effective closure of the *av* valves. The valves are better closed by the higher pressure, which raises the cusps just on to the opening and covers it. With such a leak any sudden variation of flow may set up *pulsus alternans*, which in this particular case must be looked upon as being caused by the valvular defect rather than by an inherent fluctuation of muscular function. But the condition occurs independently of valvular defect and with the heart-lung indicates a failing preparation. Fluctuation cannot be eliminated as a cause.

We see again in this record the discordant type of alternation with the higher c wave or ventricle element accompanying the lower pressure and therefore certainly not due to greater push against a sealed valve. There are many features of similarity to the clinical record above, with this difference, that in the latter we have abnormal cycles throughout but of a different degree, whilst in the experimental record normal alternate with abnormal. While in the clinical instance fluctuation of function may be the main cause of the condition it is yet possible that in it too, a mechanical factor is involved. It is natural to look upon the peculiar relation of greater leak and lower pressure as in some measure being one of effect to cause, the leak being diminished by better floating of the valves with larger volume of blood in the ventricle in the other beats. Although the difference may be slight as it is in the experimental case it may be just sufficient to upset the balance between push and pull in closure. In the clinical case, looked on in this way, a sudden increase of flow from auricle to ventricle may set up *pulsus alternans*, by tending in a given cycle to close the aperture to smaller dimensions and thus leaving a larger volume for output. A reduced flow in the next beat or two would mean greater back flow and again a larger volume in the following beat and so on. Exercise and food caused on another occasion not typical alternation but irregularity with premature beats, etc. (Fig. 7). Premature beats in experimental lesions always show early regurgitation, and this sometimes whether the opening in the tube be uncovered or not.

The auriculo-ventricular valves do not grip the tube and leave a leak beside it. It is hard to understand then how the premature beat or extra systole could be a factor in compensation as Schwartz(6) suggested.

After this paper was sent for publication, it was suggested to me that the deflection in the venous clinical record which I have described as a compound *a* and *c* wave, is a purely auricular element or exaggerated *a* wave. The suggestion appears to me untenable on the following grounds: (1) there was a shift of the *C* wave, with shortening of *A–C* interval, to the left of the apex line (cp. Fig. 2), and further shortening occurred with food and exercise until *C* ran into *A* (Fig. 3); (2) the form of the deflection with two different degrees of slope shows the presence of two factors; (3) the suggestion is not in accord with the variation of auricle beat as seen in carotid record, the stronger auricle contraction coinciding with the smaller wave; and (4) it is not in accord with change of output, the smaller output accompanying the bigger wave.

Summary.

Some normal and abnormal optical records of the heart, venous and arterial pulses are shown from human beings, and from the heart-lung preparation of the dog.

Records of mitral regurgitation in man offer evidence of leakage occurring in the early or so-called presphygmic period of systole. Those of artificial lesion in the dog afford similar evidence.

The volume of fluid regurgitated is in some measure accountable for the time in which back flow occurs, and this is especially so when the difference between auricular and ventricular pressures is marked.

Some evidence is provided in support of the view that the auriculo-ventricular valves are finally scaled as a result of the rise of ventricular pressure.

A mechanical explanation of the condition of *pulsus alternans* by alternating efficiency of the mitral valve is suggested without prejudice to the view that it may occur from inherent fluctuation of muscular function.

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(9) Wiggers. Amer. Journ. Physiol. 56. p. 415. 1921.
(10) Wiggers and Feil. Heart, 9. p. 149. 1922.

More specifically the elastic body theory in its original form states that if a muscle is able to shorten to a length l, under tension T, then if stimulated at a length l it will develop a maximum tension T. In other words the maximum tension of the stimulated muscle is dependent only upon its length (compare an elastic band). This is true for a muscle stimulated with a tetanising current of sufficiently long duration to permit it to come into equilibrium with its load. The time occupied, however, by the muscle in passing from a given length and tension characteristic of the muscle as a resting elastic body to a length and tension characteristic of it as an active elastic body (under prolonged stimulation) varies with the mechanical conditions. Hence it happens that for twitches or short tetani, relaxation may set in before the transformation is complete. In other words a muscle, which in a twitch or short tetanus can develop a tension T at length l (isometrically), may not have time to shorten to length l under a tension of T (isotonic). This is a particular case of the more general proposition demonstrated by v. Kries(4), Fick(5), Blix(6) and Seemann(7) (cf. also Bethe(8)) that tension is not a simple function either of length alone, or of length and time (after stimulation). v. Kries in particular had the idea that the mechanical changes in contraction actually affected the rate of the chemical processes underlying the contraction, but his evidence for this conception was hardly conclusive. These facts in themselves largely disprove the original elastic body theory, or at least they necessitate so many qualifications that it loses its usefulness as a simple means of analysis. Fick(9) alone among previous writers has doubted the fundamental conception at the root of the theory which postulates a development of elastic potential energy at the moment of stimulation, at the expense of which work can be performed so long as this new elastic state is maintained. My own experiments confirm Fick in this respect and suggest that a stimulated muscle, although under considerable tension, may possess very little elastic potential energy, and that the energy necessary for shortening (*i.e.* for work) is liberated as the shortening proceeds. Thus, in order to lift a weight to a given height and to perform work, W, it is not enough to liberate energy A to set up the tension and energy Bt to maintain it during the shortening; in addition, an extra amount of energy kW must also be mobilised. It is hard, therefore, to escape from the conviction that the actual motive power is kW.

The old and the new theories of the nature of muscular activity in the performance of work may be exemplified by the following two methods of lifting a weight: (*a*) work may be done against a spring,

which may then be allowed to shorten and lift the weight; (b) the weight can be raised directly by means of a chain and windlass. In the latter method every link of the chain which is wound up involves the expenditure of so much energy at the moment of winding. The chain may be under great tension, but being inextensible it possesses no potential energy. From the point of view of the energy exchange the shortening of a muscle appears to be analogous to the windlass and chain rather than to the elastic band or spring, although it may partake to a limited extent of the characteristics of the latter.

2. *Experimental analysis of the excess energy*, kW.

Under the experimental conditions previously described (2), a muscle which liberated excess energy, kW, in the performance of work, W, both raised and lowered the weight, so that the work W reappeared as heat in the muscle in relaxation. It was conceivable therefore that part (C) of kW is due to shortening in contraction, and part (R) due to lengthening in relaxation: thus,

$$E = I + kW = I + C + R \qquad \ldots\ldots(3).$$

Two methods will now be described by which C and R can be directly and separately determined.

A. *The measurement of* R *directly and of* C *by difference.* The method consists, in general, in measuring the variations in total energy output caused by lowering increasing weights. More specifically, it involves two series of observations, (a) and (b), taken alternately with varying loads.

(a) The muscle is stimulated with a short tetanus and shortens a fixed distance, s, isotonically, under tensions T_1, T_2, T_3, etc., lowering the corresponding weights in relaxation. The variations in heat observed are thus due to both C and R. The total heat recorded on the galvanometer represents the total energy liberated, E_a, and

$$E_a = I + (C - l) + R \qquad \ldots\ldots(4),$$

l is that small fraction of the energy W of the weight falling in relaxation, which is not absorbed by the muscle as heat but is wasted as heat in the apparatus by friction and impact. Measurements show that this is small enough to be neglected (cp. Table IV).

(b) The muscle, previously slightly stretched, is caused to shorten the same fixed distance, s, just before stimulation and under no tension, and to lengthen in relaxation under varying tensions, T_1, T_2, T_3, etc. Under these circumstances there is no energy change associated with the

shortening, except for the small loss, p, of potential energy by the resting muscle, and a thermoelastic cooling, e, as shown by Hartree and Hill (10). In relaxation the energy W of the falling weight is absorbed as heat by the muscle. Hence the total energy, E_b, measured as heat is:

$$E_b = (p - e) + I + R + (W - l) - (p - e) = I + R + (W - l) \quad (5).$$

The quantity $(p - e)$ appears both in the shortening and in the lengthening process, but with opposite signs so that it cancels out. I, the average of the isometric heat in both positions, can be determined. Hence, knowing E_b and W, the value of R can be calculated. Knowing R and I, C can be determined from E_a in equation (4). It should be noted that in case (a), equation (4), the energy W, although appearing in relaxation, is actually expended by the muscle in contraction and is thus a part of C. In order to compare E_a and E_b it is necessary that the stimulus, initial length, initial tension and amount of shortening allowed be the same in all cases. Hence after-loaded limited contractions have been used. For the observations of E_b a magnet was arranged to raise the weight to the fixed height just before stimulation, and to return it to the muscle for lowering, so to speak, when the muscle tension had reached its maximum. The muscle, therefore, is stimulated at its shorter length and "discovers" in relaxing that it must lower a weight. By permitting the muscle to shorten just before stimulation the correction $(p - e)$ can be made to cancel out, so that it does not record on the galvanometer.

The special lever used in these experiments and in many of those reported previously is shown in Fig. 1. It is essentially an ordinary lever of the first class; the weight is hung 7 mm. from the fulcrum at one end and the muscle pulls down 28 mm. from the fulcrum at the other. By means of the screws S and L under opposite ends of the lever the amount of shortening or lengthening of the muscle can be confined within the desired limits. The attachment, T, for releasing the lever is not used in this experiment. The only other feature of the apparatus which is essential for the present purpose is the bell magnet above the lever. The electric circuit through the magnet was *closed*, however, not as shown in the figure but by a special key on a revolving drum, so that the moment of closure could be timed to within ·005 second in relation to the moment of stimulation. When the circuit is closed, the weight end of the lever is lifted rapidly until the lever strikes the screw S. The muscle is thus permitted to shorten under no load. When the circuit is broken by the opening of another key, the weight becomes free to fall and stretch the muscle until the lever hits the screw L. The whole lever is mounted on a

stand with a screw adjustment, so that its height above the muscle can be regulated.

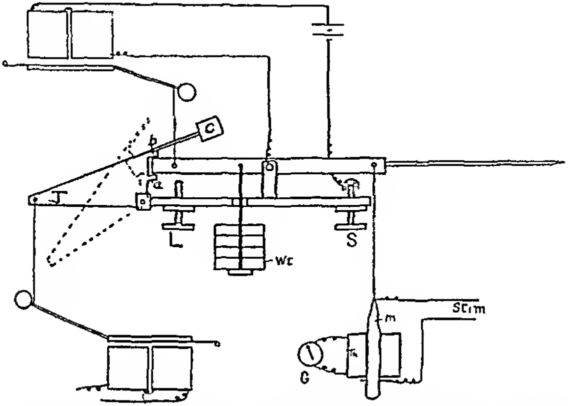

Fig. 1. Muscle lever and attachments The lever is of light brass with a bamboo writing tip The support is also of brass. *m* represents the muscle attached to the lever and resting on the thermopile, *Th*, which is connected to the galvanometer, *G* The stimulating electrodes, *stim*, are applied to either end of the muscle *C* is a counterpoise which holds the movable attachment, *T*, in position against the lever where the shoulders, *a* and *b*, serve to prevent the weight from falling or rising respectively, according to the desire of the experimenter. On the screw, *S*, is an ebonite cap bearing a contact point which makes electrical contact with the lever when it is pulled down and thereby operates the bell magnet above the lever. The brass release mechanism, *T*, is operated by the bell magnet below the lever The weight, *wt*, is hung on a wire passing through a hole in the support. *L* and *S* are set screws (cp also Text)

In all six complete experiments of this type have been performed[1]. A typical one is shown in Fig. 2. The experimental points E_a and E_b (equations (4) and (5)) are indicated by circles. The isometric heat is given in both the long and the short positions. The average may be used in the calculations. It should be noted that the axis of abscissæ in the figure does not represent the zero ordinate, and hence that the excess heat, kW, is really fairly small compared to the isometric. The excess heat, kW, is represented by the vertical distance between E_a and the isometric; it consists of two parts C and R associated with contraction

[1] In addition positive values for $(E_a - E_b)$ for single weights have been found in a considerable number of other experiments

and relaxation respectively. Part of C is W, the mechanical work performed. The relative values of C, W and R are dependent upon the

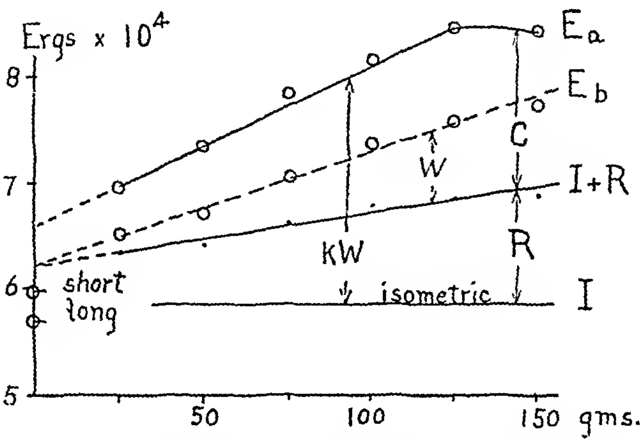

Fig. 2. Variations of C, R, kW and W with variations in load. kW is the excess energy for the combined process of raising and lowering; W is the work; C and R are the fractions of kW associated with contraction and relaxation respectively. Duration of stimulus 0·2 second. Weight of muscle 0·33 gm. Temp. 0° C. Muscle shortens 2·4 mm. in each case.

accuracy of the calibration but the rather considerable variations observed in different experiments, particularly in the values of R, are too great to be explained in this way. The fact, however, that $E_a > E_b$ is entirely independent of the calibration, and proves that in shortening under a load the muscle liberates as heat a certain amount of energy $(C - W)$ in excess of the isometric, in addition to the energy, W, which is stored in the weight as potential energy. The value of $(E_a - E_b)$ or $(C - W)$ increases slightly with the load, but does not diminish to zero at zero load if the dotted prolongations of E_a and E_b are correct. This suggests that a considerable part of $(C - W)$ is associated with the frictional loss, f, involved in the change in shape of the muscle. If $(C - W)$ equals f then the simple conclusion can be drawn that the excess energy, kW, associated with contraction is equal to the sum of the external work, W, and the internal work, f. The difference between E_a and E_b can hardly be due to the fact that the muscle is stimulated in the long position in the former and in the short position in the latter because, to judge from the isometric heats in the two positions, E_b should then be larger than E_a. The fact that the reverse is true at zero loads indicates that shortening without tension involves a greater expenditure of energy than tension without shortening.

The most important new point in connection with Fig. 2 is the existence of R, the excess energy associated with the lowering of the weight in relaxation. The existence of such an effect has occasionally been debated (Frank (11)) but no convincing experiments have been forthcoming, chiefly on account of the difficulty of expressing both heat and work in the same units. To verify this result I have conducted on 14 muscles 24 series of observations similar to those in the E_b series, Fig. 2, in which the conditions of stimulation and contraction have been constant, while the loads lowered in relaxation have been varied. The work, W, done on the muscle by the weight in falling also has been calculated, and E_b has been found to increase more rapidly than could be accounted for by the increase in W. Thus $(E_{b_n} - E_{b_1}) > (W_n - W_1)$, where E_{b_1} and W_1 apply to the smallest weight used and E_{b_n} and W_n to any larger weight This result has been obtained invariably in ten out of the 14 muscles used: it is perhaps significant that I have never failed to obtain it except during the summer months when the temperature was higher and the frogs were in very poor condition.

In Table I the results of four typical experiments of this sort have been collected, all of which show an extra liberation of energy involved in lowering the various weights. It should be noted that this excess is calculated on the assumption that $R = 0$ with the smallest weight. Both

TABLE I. Increased heat due to relaxing under increasing weights

Exp. A

Increase in work $W_n - 21$	Increase in heat $E_{b_n} - 693$	$E_{b_n} - 638$
14	44	52
28	73	75
42	120	116
56	145	—
—	—	—

0 31 gm.; 0° C.

Exp B

Increase in work $W_n - 15$	Increase in heat $E_{b_n} - 729$	$E_{b_n} - 728$
15	21	20
31	60	51
46	85	102
61	135	116
67	147	—

0 26 gm , 8 2° C

Exp C

Increase in work $W_n - 47$	Increase in heat $E_{b_n} - 2040$	$E_{b_n} - 1340$
38	159	80
75	345	218
113	384	233
150	449	—

0 54 gm , 9 7° C

Exp D

Increase in work $W_n - 13$	Increase in heat $E_{b_n} - 910$	$E_{b_n} - 798$
14	3	30
41	81	89
68	113	108
95	162	—

0 17 gm ; 15° C

Both heat and work are given in units of 100 ergs The muscle was stimulated for 0 2 second in each case with a current of suitable strength from the alternating current mains The weight of each muscle and the temperature are given under each experiment Two series of observations with each muscle are recorded Each of these is the average of two readings, one in the ascending, the other in the descending order of weights

Fig. 2 and Fig. 3 make it probable that $R > 0$ even for zero loads, so that the values of $(E_{b_n} - E_{b_1}) - (W_n - W_1)$ from Table I are probably less than the true value of R. The first column shows the increase in work with increasing weights, above that involved in lowering the smallest weight, *i.e.* $W_n - W_1$. In this case the work is done by the weight upon the muscle. The second and third columns give the corresponding increase in heat $E_{b_n} - E_{b_1}$ as observed in two duplicate series. The second and third columns are, with a single exception, larger than the corresponding values in the first column. The effect of fatigue becomes evident when the two values of E_{b_1} for each experiment are compared. Fatigue was least in Exp. B, E_{b_1} decreasing from 729 to 728 only, and was greatest in Exp. C, where E_{b_1} fell from 2040 to 1340. Because of fatigue the observation with the highest weight in each second series had to be omitted. The existence of R cannot be explained by an error in the calibration, for R is usually larger than W and a 100 p.c. error in the calibration is impossible.

The interpretation of R, the excess heat associated with lengthening of the muscle in relaxation, is difficult but it is probably misleading to regard it as an expression of an "effort" made by the muscle in lowering the weight. It may be suggested rather that it is best correlated with the time at which lengthening begins. Thus the larger the weight the earlier in relaxation the stretching begins and the greater the tension of the muscle during the stretching. It is because of this greater tension with the larger weights that the muscle is able to absorb as heat all the energy liberated by the various weights in falling.

In a previous paper (2) it was found that the excess energy involved in work on an isotonic lever was 1·8 times the work performed, while the corresponding figure for an inertia lever where the work was not re-absorbed as heat in relaxation was only 1·3. This difference is doubtless due to the excess energy liberated in lowering the weights in relaxation and is good indirect evidence in support of the experiments just described. Thus $1{\cdot}3W + R = 1{\cdot}8W$. The figures are approximate only.

B. *The measurement of* C *directly and of* R *by difference.* This method consists essentially in letting the muscle lift increasing weights through the same interval. After being lifted the weights are held up by an electromagnet so that the muscle does not lengthen again in relaxation and the galvanometer reading is taken with the muscle in the short position. The muscle is also calibrated in that position after the experiment. The electromagnet is arranged above the lever as shown in Fig. 1, the circuit being closed as soon as the lever makes contact with

the insulated metal cap on the screw S^1. This circuit is broken again by hand after the galvanometer deflection is taken.

In an experiment of this sort the heat, H, recorded by the galvanometer is given by the formula:

$$H = I + C - W + (p - e) \qquad \text{......(6).}$$

The energy, W, actually used in lifting the weight is stored in the weight as potential energy and does not appear in the muscle as heat. The total energy, E, liberated by the muscle in the contraction therefore is

$$E = H + W - (p - e) = I + C \qquad \text{......(7).}$$

Usually H turns out to be roughly constant, indicating that the increase in heat with increase in load is just equal to the increase in work done. It does not mean, however, that the total excess heat, C (associated with the shortening), is equal to the work done, W. It may he observed that part or all of the energy $C - W + p$ has been expended by the muscle in changing its own shape, *i.e.* in doing work, f (frictional loss) upon itself, which of course appears finally as heat in the muscle. So far it has not been possible to measure f directly in the stimulated muscle but it does not seem impossible that $C = W + f - p$, *i.e.* that the excess energy of the contraction phase is equal to the total work, external and internal, which is performed, after making the small correction, p, for the potential energy lost by the resting muscle in shortening.

Data of this sort are given in Table II. For each experiment the work, W, done by the muscle in lifting the various weights and the corresponding amounts of heat, H (equation (6)), liberated as such in the muscle have been tabulated. The total energy, E, is equal to the sum of the work and the heat, minus the small correction $(p - e)$. The shortening of the muscle was about 3 mm. in every case. In all these experiments the heat remains practically constant, or at least the variations of heat are small compared to the variations in work done. There is a fairly well-marked tendency for the heat to decrease with the highest weights. Later experiments with certain of these same muscles, when they were more fatigued, showed a decrease in heat with increase in weight. Two other muscles showed a decrease in heat even when first dissected. This also may have been an effect due to the poor condition of the muscle, for many of the muscles were unfit to use even when first put on the thermopile. The work in these exceptional cases must have been performed, in

[1] The kinetic energy of the weight when the lever hits the screw S and makes electrical contact has been measured from its velocity as obtained from records on a moving drum. It is so small that it has been neglected.

part at least, by the energy mobilised in an isometric contraction. In fatigue the whole mechanism by which excess energy is liberated for the performance of work seems to be eliminated, as I have observed in many experiments.

TABLE II. Heat production when varying loads are lifted a constant height.

Exp. A

Work	Heat *a*	Heat *b*	Heat *c*
0	117	92·1	63·6
1·0	116	91·4	66·5
2·1	117	93·8	67·2
3·2	124	—	—
4·2	119	93·4	66·4
6·2	125	89·9	61·4
8·4	115	92·9	—
10·5	120	90·6	—
12·7	117	89·6	—
14·8	118	—	—

0·2 sec.; 12·7° C.; 0·27 gm.

Exp. B

Work	Heat *a*	Heat *b*	Heat *c*
0	71·9	214	98·9
1·3	74·4	—	—
2·7	74·8	214	98·6
5·4	73·7	217	99·1
8·1	70·4	219	102·2
10·8	—	219	—
13·5	—	212	—
16·2	—	209	—
—	—	—	—
—	—	—	—

0·05 sec. (*a* and *c*); 0·2 sec. (*b*); 14° C.; 0·22 gm.

Exp. C

Work	Heat
1·4	98·4
2·5	100·8
5·4	100·5
8·1	99·3
10·9	97·7
—	—

0·2 sec.; 15° C.; 0·12 gm.; isom. $88·5 \times 10^3$ ergs

Exp. D

Work	Heat
0·7	94·3
2·1	95·4
4·8	94·8
7·5	94·1
10·2	95·7
12·9	91·9

0·2 sec.; 15° C.; isom. $93·4 \times 10^3$ ergs

Exp. E

Work	Heat
5·0	215
12·8	219
19·6	226
26·3	224
—	—
—	—

0·2 sec.; 17° C.; isom. 205×10^3 ergs

Work and heat are given in units of 1000 ergs. Duration of stimulus, temp. of the muscle, weight of the muscle between electrodes are given where possible. The isometric heat where given applies to the short position of the muscle. Each figure is the average of two series taken in ascending and descending order of weights respectively. In A and B the initial tension was greater than 0 and the observations with zero work were obtained by preventing shortening until the moment of stimulation by the method described below in §§ 4 and 5.

In three of the experiments of this type simultaneous observations were made of the energy, E_a, necessary to lift and drop the same weights. These readings alternated with those taken when the weight was not dropped (as in Table II) so that the muscle first lifted the weight, then lifted and dropped it. A larger weight was then hung on the lever (after-loaded) and the procedure repeated. A typical experiment is plotted in Fig. 3 and is instructive in showing the inter-relationships. In the upper curve values of E_a (equation (4)) are plotted as in Fig. 2.

The lower curve gives values of the heat, H (equation (6)), recorded when the weight was not lowered in relaxation.

The middle curve gives the values of $H + W$

$$H + W = E_c = I + C + (p - e) \qquad \text{......(8).}$$

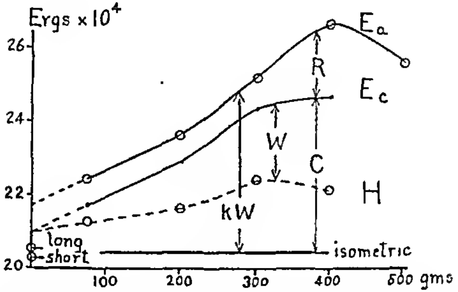

Fig. 3. Similar to Fig. 2, but by a different method. Variations in load on the excess energy, kW, and its two fractions C and R, associated contraction and relaxation respectively. Experimental points are indicated by circles. Duration of stimulus, 0·2 second. Weight of muscle 0·27 gm. Muscle shortens 2·7 mm., *i.e.* from 103 p.c. to 93 p.c. of its extended length in the frog. Temp. 18° C. Abscissæ represent weights on the lever. The corresponding tension on the muscle during shortening was a quarter as great.

In order to correct for $(p - e)$ measurements were made of the galvanometer deflection obtained when the resting muscle was stretched by the smallest weight (75 gms.). Contrary to the theoretical expectations a slight cooling, $0{\cdot}25 \times 10^4$ ergs, was observed. In E_a the muscle has lengthened; in E_c and H it has not. Hence the cooling due to lengthening has been deducted in the figure from values of E_c and H. Actually the correction should have been slightly larger (thus increasing R slightly) because in measuring it the muscle must have been heated somewhat by the energy of the falling weight, *i.e.* $W - p - l + e$. Thus the correction $p - e$ is automatically taken care of. This method of correction will be analysed in more detail below.

It is evident that the experiment in Fig. 3 serves the same purpose as that in Fig. 2, in making possible a subdivision of the excess energy, kW, above the isometric into two fractions, C associated with the lifting of the weight and R associated with the lowering of the weight. Now C is obviously larger than W, even at zero load, if the dotted extrapolations are justified. $C - W$ at zero load should represent the work done internally in changing the shape of the muscle, *i.e.* frictional loss, f. This

figure agrees with Fig. 2, therefore, in lending support to the possibility that $C = W + f - p$ as suggested above.

In Fig. 3, unlike Fig. 2, there is more isometric heat at the long position than the short. The values of E_a pass through a maximum, decreasing at the highest weight. This fall was accompanied by a fall in the work which is not shown in the figure.

3. *Energy liberation when work is done upon the muscle by a falling weight during the contraction period.*

It has been shown that when positive work is done by the muscle there is a positive excess heat liberation, C, associated with the shortening period. If this represents a fundamental process in the muscle economy, the converse should also be true. Thus when the muscle does negative work the excess energy C should also be negative.

The performance of negative work by the muscle can be arranged by stretching it so that s becomes negative in the formula $W = Ts$. The method employed, in principle, is to hang on the muscle a weight which is supported previous to stimulation but is released shortly afterwards and allowed to fall a fixed distance, thus stretching the muscle a distance s. By properly varying the size of the weight and the time of release the muscle can be stretched at any time during contraction or relaxation. If a small weight is released when the muscle tension is near its maximum it will not fall until toward the end of relaxation. Larger weights will fall earlier in relaxation. A weight too heavy for the muscle to lift will fall as soon as it is released in spite of the "contraction" of the muscle. Thus the abnormal process of stretching during the contraction period can be made to grade imperceptibly into the normal process of relaxing under different loads. The energy, E, liberated in the muscle as heat as a result of the physiological processes of contraction is determined by deducting the energy, W, put into the muscle by the falling weight from the total heat, H, found in the muscle by the galvanometer. If it is true that negative work causes negative excess heat, then it is to be expected that, when the stretching takes place during stimulation, W will not add itself to H but will to some extent replace H.

In practice this experiment is carried out by means of an attachment to the lever which is shown in Fig. 1. At the end of the lever opposite to the muscle is a triangular piece of brass, T, held in position by a counterpoise, C, and carrying two shoulders, a and b. Only the lower of these, a, is used for this experiment; it serves to support the lever before its release. When the circuit is closed through the electromagnet below

the lever, the magnet pulls upon T by means of a connecting thread and releases the lever. The weighted end of the lever then falls until it hits the screw L, thus stretching the muscle and recording the moment when stretching begins on a revolving drum. The electric contact on the screw S is not used for this experiment. S is so adjusted that it just touches the lever when the other end of the lever rests upon the shoulder a, thus preventing the least bit of shortening in the muscle until it is released. If not prevented by the tension of the muscle the weight begins to fall ·03 to ·04 second after the circuit through the electromagnet is closed, this being the mechanical delay in the releasing apparatus. The circuit is closed automatically by an arm and a knock-down key on the same revolving drum which is used for the timing of the duration of the stimulus. The moment of release in relation to the beginning of stimulation is thus known to within ·005 of a second. Short tetani of 0·2 second have been used. The source of current was a rotating commutator rapidly reversing a direct current or a small A.C. bicycle dynamo (Downing(12)).

Before describing the experimental results the energy changes in the muscle during the experimental procedure must be analysed in more detail. The heat, H, developed in the muscle and measured on the galvanometer is given by the formula

$$H = I + C \text{ (if any)} + R \text{ (if any)} + (W - l) - (p - e) \text{......(9)}.$$

As before p is that portion of W which goes to restore the resting potential energy of the stretched muscle; l is that portion of W which is not absorbed by the muscle but wasted as heat when the lever strikes the screw L. The energy, E, liberated physiologically by the muscle is given by the formula:

$$E = I + C + R = H - (W - l) + (p - e) \quad \text{......(10)}.$$

The quantity of physiological interest is E, obtained by adding $p + l - e$ to $H - W$. Now $p + l - e$ is in any case small and does not vary with the weight and time of stretching so that the relative values of $H - W$ are significant without this correction. But in order to compare the isometric heat in either position, I_s or I_l with $H - W$, it is necessary to know $p + l - e$. I have sought to determine this by measuring on the galvanometer the heat, g, produced when the smallest weight is allowed to stretch the resting muscle a distance s under tension T, the potential energy lost by the weight being $Ts = W_1$. The value of g should then be $g = W_1 - p - l_1 + e$. Now $W_1 > p + l_1$. Hence g should be positive. Actually it was negative which must indicate a cooling, $-c$, due pre-

sumably to minute differences of temperature along the muscle. This new factor must be introduced into the above equations as follows:

$$g + W_1 = p + l_1 - e + c,$$

$$E = H - W + p + l - e + c.$$

Hence $$E = H - W + (g + W_1)^* \qquad \text{......(11)}.$$

In other words, simple lengthening of the unstimulated muscle causes a cooling so that $H - W$, as observed, is too small by this amount. The necessary correction, $g + W_1$, has been determined in three of the six experiments in Table III, and its value has been found to be too small to affect the sense of the results although it mars their technical perfection.

Some of the results of ten series of this sort are collected in Table III. The work is calculated directly in ergs. The heat is calculated as ergs from the galvanometer deflection by means of the electrical calibration of the muscle. The calibration was usually taken in the long position. The isometric heat in the short position was obtained by means of an additional calibration in that position.

TABLE III. The decrease in the energy liberation caused by stretching the muscle during the contraction period.

	Work done on the muscle				Energy liberated by the muscle					
Exp.	W_1	W_2	W_3	W_4	H_1-W_1	H_2-W_2	H_3-W_3	H_4-W_4	Isom. *s*	Isom. *l*
1	27	163	326	380	1633	1769	1509	1414	1726	1761
2	13	109	326	326	725	671	468	408	784	663
2 *a*	13	81	272	326	609	555	380	354	554	570
3	13	109	326	—	1013	1028	818	—	(941)	1012
3 *a*	13	109	326	—	1068	1122	1015	—	—	1069
4	13	109	217	—	846	940	682	—	—	861
5	13	109	271	271	560	597	499	461	—	—
6	13	109	271	—	1012	995	853	—	—	—
6 *a*	13	109	271	271	1014	950	812	800	1056	994
6 *b*	13	109	271	—	941	921	753	—	—	—

All quantities are expressed in units of 100 ergs. The value of the correction $g+T_s$ or $g+W_1$ was 98, 33 and 40 × 100 ergs in Exps. 1, 2 and 3 respectively. All muscles at room temp. about 15° C. Amount of shortening was 2·2 mm. throughout. Isom. *s* and isom. *l* = isometric heat in short and long positions.

Each complete experiment started with an isometric contraction in the short position. The smallest weight, W_1 (25 gms. in Exps. 2–6; 50 gms. in Exp. 1), was then hung on the lever, supported in the short position until released at about the end of stimulation. With this weight the muscle was stretched toward the end of the relaxation period (0·4 second after stimulation). Larger weights were then used in the same

* This calculation is not strictly accurate because the value of l varies with the load, and l_1 for the unstimulated muscle is not necessarily equal to l for the stimulated muscle.

way until the muscle was stretched as soon as the weight was released at about the end of the stimulation (0·2 second). W_2 in Table III represents a weight, usually 200 gms. which was just large enough to stretch the muscle at 0·3 second after the beginning of stimulation, and which was approximately the largest weight under which the muscle could shorten as much as 2·2 mm. W_3 represents a weight (500 or 600 gms.) large enough to stretch the muscle as soon as it is released, *i.e.* between 0·1 and 0·2 second after the beginning of stimulation. W_4 is a weight which was released sooner and began to stretch the muscle in less than 0·1 second after the beginning of stimulation. W_1 and W_2, therefore, represent normal relaxations; W_3 and W_4 stretch the muscle during the contraction period when the muscle should be shortening. Other intermediate weights were also used in addition to those included in Table III. After the observation with the largest weight the muscle was stimulated isometrically in the long position and then the various weights were used again in the reverse order. The results of the two observations with each weight were averaged in order to eliminate the effects of fatigue.

The results show that less energy is mobilised by the muscle when it is stretched during stimulation ($H_3 - W_3$ and $H_4 - W_4$) than when the same lengthening is carried out in relaxation ($H_1 - W_1$ and $H_2 - W_2$) or than when the muscle is similarly stimulated isometrically in either the long or the short positions (I_s or I_l). The result is most evident in $H_4 - W_4$ where the stretching took place most nearly at the moment when the tension was being increased. In the first experiment, I_s and I_l are 172,600 and 176,100 ergs respectively. It might be supposed that, if the muscle changed from one length to the other at any moment during the contraction or relaxation period, the resulting energy liberated would be intermediate between the isometric heats for the two positions. This is not the case. If the change is from the long to the short position during the contraction period the energy liberated is greater (*i.e.* C and W are both positive) than either I_s or I_l as already shown. Table III shows that if the change is in the opposite direction from the short to the long position that the heat liberated is considerably less than I_s or I_l, *i.e.* C and W are both negative. Thus $H_4 - W_4$ (Exp. 1) or 141,400 ergs plus the correction 9800 ergs = 151,200 ergs, which is less than 172,600 or 176,100 ergs. When 38,000 ergs work is done upon the muscle in stretching it, the energy liberated is decreased 176,100 − 151,200 ergs = 24,900 ergs. The work done in stretching the muscle does not therefore add itself to the "physiological" heat but 249/380 or 65 p.c. of it (in this case) replaced energy

which would have been liberated by the muscle if it had not been stretched. Again in the same experiment comparing H_3 and H_4 it is found that when 32,600 ergs work, W_3, is done upon the muscle the heat observed is 150,900 + 32,600 = 183,500 ergs; and when, by stretching the muscle earlier after the beginning of stimulation, 38,000 ergs work, W_4, is done upon the muscle the heat observed is only 141,400 + 38,000 = 179,400 ergs. In the latter case the heat observed is 4100 ergs less than in the former although 5400 ergs more work was done upon the muscle and appeared within it as heat. This is perhaps a particularly striking case but it is in agreement with the general trend of all ten series as well as with four others not sufficiently complete to tabulate.

That inappreciable fractions of the energy of the various weights were dissipated as heat in the apparatus is shown in Table IV. This gives for the various values of W the corresponding values of the kinetic energy of the weight, $l = mv^2/2$ just at the moment when the lever hits the screw L, and the times after the beginning of stimulation when the muscle began to lengthen. At most 10 p.c. of the energy of W may be wasted in the apparatus.

TABLE IV. Energy waste in apparatus and time of lengthening with varying amounts of negative work, W.

Work	Energy waste, l	Time when lengthening begins in seconds
13	0	·58
27	1	·35
54	4	·32
109	11	·30
163	12	·28
216	20	·25
326	10	·15
326	0	·10
326	0	·07
326	0	·03

W represents the work in units of 100 ergs done upon the muscle by weights (on the lever) varying from 25 to 600 gms. The energy waste is the kinetic energy of the weight in ergs × 100 at the moment when lengthening is complete and represents that fraction of W which was not absorbed by the muscle as heat. The time when lengthening begins is measured from the beginning of the stimulus (0·2 second) and was obtained from records on a moving drum. In the last four, lengthening began in each case ·03 second after the closure of the electric contact which operated the releasing mechanism. Data taken from Exp. 2, Table III.

The experiments in this section show that stretching of a muscle during the contraction phase causes a decrease in the heat production. This may be interpreted as due to the performance of negative work by the muscle. Positive work increases the heat production; negative work decreases it.

4. *The heat liberated when a muscle is prevented from shortening until some time after stimulation.*

The experiments fall into two groups. In the first. the muscle lifts a weight as it shortens and is pulled back to its original position by the weight in relaxation. In the second group, considered in section 5, no external work is done in shortening and the muscle is not pulled back to its original position in relaxation.

In order to carry out these experiments it is necessary to hold the muscle isometrically for various times after stimulation and to release it at a desired moment. This is accomplished by the release mechanism T, and the shoulder b, attached to the lever (Fig. 1). The screw L is arranged so that the lever is held snugly between it and the shoulder b. The screw S is then adjusted to permit the desired amount of shortening; or it is screwed down out of the way altogether. At the desired moment the circuit through the bell magnet under the lever is closed automatically; T is pulled down and the lever released.

In the first type of experiment the initial tension is constant and small (6–12 gms.). The weight, if greater than that necessary to set up the initial tension, is after-loaded. The muscle is first stimulated and allowed to shorten freely. The initial isometric interval is then increased until the whole contraction becomes isometric. The results are averaged with a similar series of observations taken in the reverse direction to avoid errors due to fatigue. The result of an experiment is plotted in Fig. 4. The muscle was stimulated for 0·4 second. The height of the contraction was not limited and the amount of shortening progressively decreased until the contraction became isometric. Abscissæ represent the time of release of the lever, no allowance having been made for the time (·04 second) consumed in effecting this release after the circuit was closed. At zero time when the muscle was not restrained at all the heat production was considerably greater than the isometric, as was to be expected. There is then a very slight increase in heat which does not always appear and may be due to a somewhat higher tension in the muscle when the shortening begins. With further increase in time the heat and work both decrease to the isometric point. For mechanical records of the changes of length of muscles carrying out contractions of this sort, "mit Anfangshemmung," see the figures of v. Kries (4) which show that the greater the isometric interval the more the completion of relaxation is delayed.

The fall in heat in Fig. 4 may be due to a decreased shortening and

hence decreased work. In order to show that the *time* when shortening takes place is also a factor, similar experiments have been carried out

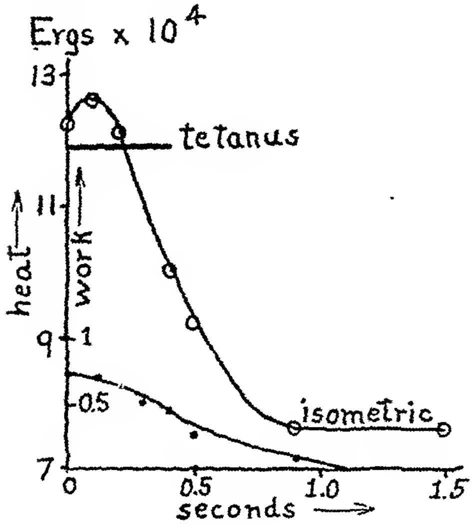

Fig. 4.

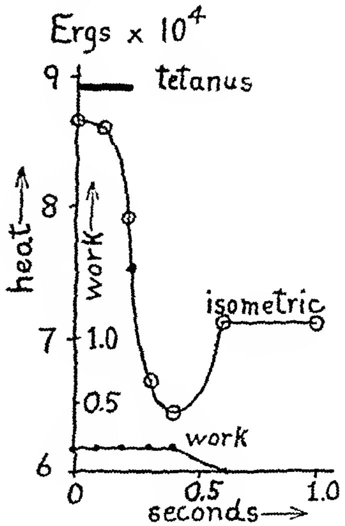

Fig. 5.

Fig. 4. Decrease in heat production and work caused by preventing a muscle from shortening for increasing periods *after the beginning of stimulation (see Text). Temp. 0° C.* Weight of muscle between the electrodes was 0·33 gm. Lever weighted with 125 gms. Tension on muscle during shortening $=\frac{125}{4}$ or 31 gms. Initial tension 6·2 gms.

Fig. 5. Variation in heat production (ordinates) with increase in the time after stimulation when shortening begins (abscissæ). Similar to Fig. 4, except that the shortening was *limited in every case to 2·7 mm. Muscle at room temperature, 8° C. Weight of muscle,* 0·26 gm. The lever was weighted with 25 gms. giving a muscle tension of 6·2 gms.

except that the degree of shortening was limited in each case to 2·7 mm. It is thus possible to determine the effect of the *same* shortening under the same tension (*i.e.* the same performance of work) when it takes place at different times after the beginning of the stimulus. The resulting curves are shown in Fig. 5. The general feature is the same as in Fig. 4, the heat falling off rapidly toward the isometric. It may be concluded, therefore, that the longer the isometric interval before shortening begins the less the excess heat liberation for the same work, until it fails to appear altogether. Fig. 5 shows, however, an *additional feature* in the minimum through which the curve passes. This minimum is a very characteristic feature of such experiments whether the height of contraction is limited or not, and indicates that if the muscle is released at just about the time that relaxation begins (*or at the time of maximum tension) the heat production is less than if it had been held fast in the*

original position. One is thus tempted to conclude that whereas shortening in contraction causes an increased heat production, the same shortening in relaxation (when the muscle is normally lengthening and when possibly the contractile process is in some particulars reversed) causes a decreased heat production. Conversely it has been shown (§§ 2 and 3) that lengthening in contraction causes a decreased heat production and lengthening in relaxation (usually) an increased heat production. It is too soon to speculate upon the meaning of these facts.

Summarising the experiments just described it is found that 17 curves similar to those published have been obtained on four different muscles. All showed a marked decrease of heat with increasing preliminary isometric interval; nine showed a slight initial increase in heat; 11 showed a minimum of heat when shortening occurred in relaxation. One of these muscles which served for two days of continuous experimentation on this and other points, failed to give the characteristic curve at the end of the first day when it was much fatigued. On the following day, however, when it had recovered, it gave five very good series all showing a marked decrease in heat with increasing isometric interval. The duration of stimulation in these experiments has varied from ·05 to 0·5 second. The heat does not show a decrease unless the lever is held isometrically until approximately the end of the stimulus, whether it is a long or a short one.

Through the courtesy of Mr Hartree and Prof. A. V. Hill, I am permitted also to mention some unpublished experiments performed by them on frog sartorius muscles in May and June, 1922. The experiments were similar to that plotted in Fig. 4. There were in all 22 series on seven different muscles. Sixteen of these curves resembled mine, particularly as to the minimum in the heat produced just before the contraction became completely isometric and as to the maximum in heat when the muscle was not restrained. In six series the isometric contraction gave the most heat for some reason not at present understood. The temperature varied from 0° to 15° C. and the stimulus from single shocks to 0·4 seconds tetanus. It is very reassuring to find that I have confirmed their results so well with an entirely different set of apparatus.

Similar experiments have been carried out by Fick(13) and by Schenck(14) using the inner muscles of the frog's thigh and I have been able to confirm their results in a general way on a pair of gastrocnemius muscles. In a gastrocnemius muscle the isometric heat is greater than the isotonic heat. Consequently the more nearly isometric a contraction becomes the greater the heat production. The experiments described

above show that the reverse of this is true in the sartorius. Reasons for this important difference between the gastrocnemius and the sartorius muscles have been suggested in a previous paper (2).

5. *Variations in energy liberation caused by releasing a stretched muscle at various times before and after stimulation.*

The experiments are made in the same way as in § 4 but *without a weight* and with a muscle which is initially so much stretched that it will shorten the required distance without stimulation, merely by the elasticity of the connective tissue. This modification enables the experimenter to make the muscle shorten before stimulation as well as after it without difficulty.

The essential difference between these experiments and those plotted in Figs. 4 and 5 is that in this case no *external* work is done because the shortening takes place under zero tension as far as the whole muscle is concerned. The individual muscle fibres, however, may shorten under some slight tension, if the shortening of the muscle when stimulated is more rapid than the shortening unstimulated. Thus there may be some slight *internal* work done in accelerating the change of shape of the muscle. For these reasons one might expect that the proposed modification of experiments of Figs. 4 and 5 would merely decrease the maximum heat due to the decrease in work. The results of the five experiments plotted in Fig. 6 seem to justify this expectation to some extent.

In these curves (Fig. 6) the abscissæ represent the time when shortening began, ·04 second having been allowed for the mechanical delay in the releasing apparatus. The method of release was identical with that described above (§ 4). Each point plotted is the average of two observations, one made while the time after stimulation was being progressively increased and the other while it was being decreased. Points at the extreme left of the figure represent the heat obtained by releasing the muscle 0·28 second *before* an isometric contraction in the short position. Conversely points at the extreme right show the heat liberated in an isometric contraction at the long position, followed by the usual shortening. It will be noticed that the isometric long are always lower than the isometric short showing that the muscle in the long position had been stretched beyond the optimum length for the maximum isometric heat production. Stimulation began in all cases at zero time. In the four upper curves, *A*, *B*, *C* and *D* the stimulation lasted 0·1 second and the point of minimum heat is correspondingly later than in *E*, where maximal break shocks were used.

The upper three curves of Fig. 6 are perhaps the most typical. All show a slight rise to a maximum when the shortening begins just at the

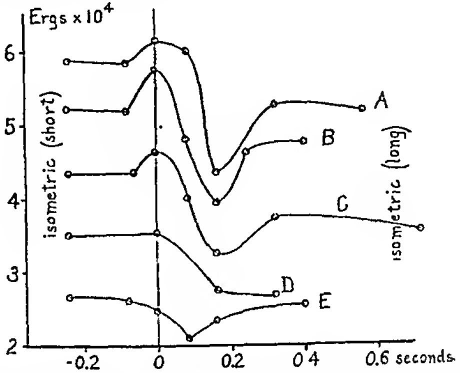

Fig. 6. Variations in heat production caused by releasing a stretched muscle at various times before and after the beginning of stimulation. Stimulation began at zero time, and lasted for 0·1 second except in E where maximal break shocks were used. E is plotted in mm. deflection since no calibration was taken. Curve A has been raised in all points 4×10^3 ergs to avoid overlapping. The shortening was 2·7 mm. throughout.

moment of stimulation. This is probably due to the slight internal work which the fibres must do in accelerating the shortening of the muscle. Evidence of this same fact has been discussed in connection with Figs. 2 and 3. Immediately following this maximum there is a minimum which is undoubtedly the homologue of the minimum obtained in Fig. 5, and in other similar experiments when the muscle shortened under a weight. Whatever the explanation it is certainly a most constant feature of such experiments and has been obtained in 20 out of 24 series with eight different muscles. The maximum corresponding to the moment of stimulation is not so common. It appeared in only 11 of the 24 series. Curves D and E are typical cases where it failed. Both of these can be attributed to fatigue of the muscles. Possibly other failures can be similarly explained because all of the experiments of this type were performed at a time when the frogs were in particularly poor condition. The same muscle which gave curve D on the fourth trial had given curve C and two other very similar ones previously. Likewise curve E was the fourth obtained with another muscle, the first of which had shown an initial small but

definite maximum. Curves *A* and *B* were also the first and fourth, respectively, obtained with one muscle. The second of these four had failed to show a maximum due to pronounced fatigue; but after a rest of one or two hours in Ringer's solution the signs of fatigue disappeared and the typical maximum was obtained in the third and fourth (*B*) series.

A. V. Hill(15) has performed experiments with the gastrocnemius and semimembranosus muscles similar to those described above, but owing probably to the anatomical peculiarities of those muscles he obtained quite different results from mine. He found that a muscle allowed to shorten freely without load immediately after stimulation liberated less heat than if shortening was prevented indefinitely or for some time after stimulation. Reasons for this difference have already been suggested (1).

Summary.

1. Experiments are described which show the effects of a small amount of shortening or lengthening of a sartorius muscle, at various times during contraction and relaxation, on the total energy mobilised in the muscle as a result of a given stimulus.

2. Shortening during the contraction period increases the energy liberated. The excess energy due to shortening in contraction is very nearly equal to the work done, if we include in that category both the external work done in lifting a weight and the internal work causing the change in shape of the muscle. There may be a slight excess heat production when the muscle shortens without a load, due to the internal work alone. The longer the shortening is delayed after the beginning of stimulation, the less is the excess energy liberated.

3. Shortening during relaxation decreases (usually) the energy liberated. If a muscle be released and allowed to shorten at or near the beginning of relaxation the energy liberated is usually somewhat less than in an isometric contraction.

4. Lengthening during the contraction period decreases the energy liberated. This is interpreted as meaning that when the work done by the muscle is negative the excess energy is also negative. The longer this lengthening is delayed after the beginning of stimulation the less the decrease in energy observed. The amount of this decrease is somewhat less than the work done upon the muscle in stretching it.

5. Lengthening during relaxation increases (usually) the energy liberated. Thus when a muscle lifts a weight in contraction and lowers it in relaxation a part (30 to 40 p.c.) of the excess energy observed is due

to processes involved in the lengthening of the muscle rather than in the shortening.

6. It is shown that the existence of an excess heat liberation is to some extent inconsistent with the idea that a stimulated muscle is a new elastic body. It is suggested that contraction is analogous rather to the winding up of an anchor chain by a windlass than to the lifting of a weight by the energy of a stretched spring.

It is a pleasure to acknowledge again my indebtedness to Prof. A. V. Hill, who suggested the work and has advised me continuously during the experiments.

A grant to Prof. Hill from the Royal Society has defrayed part of the expenses of the experiments reported in this and the previous paper. I wish to express also my gratitude to the Rockefeller Institute for Medical Research, New York City, for a travelling fellowship which made this work possible.

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In my previous paper I overlooked a publication by Bürker in *Pflüger's Arch.* 174. p. 282, 1919, in which the author described experiments similar to mine with the semimembranosus and gracilis muscles of the frog. It is to be regretted that he did not continue to increase the weights until the contractions became completely isometric. The published data are not sufficiently extensive to enable one to determine whether the muscles used by him behaved like the sartorius or like the gastrocnemius

The conception of an increase in energy liberation associated with the performance of work fits in well with the experiments of Chauveau (*C. R. Acad. d. Sci.*, 122. pp. 58, 113, 1896) who found a greater oxygen consumption in going up stairs than in going down the same stairs backwards at the same rate. Here the tension-time curve of the muscles must have been the same in both cases but in the former the loaded muscles were shortening and in the latter lengthening. The difference in oxygen consumption was equivalent to something more than twice the work done.

THE INSEPARABILITY OF THE MECHANICAL AND THERMAL RESPONSES IN MUSCLE.

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OUR knowledge of the nature of the muscular mechanism is based on observations of such manifestations of its activity as tension, heat, electrical potential change, acidity and metabolism. It would be very useful in the analysis of the mechanism of contraction if states could be found in which the functions could be independently eliminated. Several attempts to separate tension and the potential change have met with apparent success particularly in the poisoned heart. These experiments have been reviewed recently by Einthoven and Hugenholtz(1). They call attention to the great discrepancy between the energy necessary to move a galvanometer string and a muscle lever, and give some experiments, which were extended by Arbeiter(2), to show that if a sufficiently delicate and frictionless lever is employed, the tension and electrical potential in heart muscle decrease in a similar manner.

In skeletal muscle it was shown by Biedermann(3) that if water rigor is produced in one end of a curarised sartorius, this end may be electrically stimulated and produce contraction in the normal end without itself contracting. The observation was confirmed by Engelmann(4) in the heart and later in skeletal muscle by Härtl(5) under conditions which exclude the possibility of spread of the stimulating current to the uninjured muscle. The only apparent objection to these experiments is that the recording apparatus may have been insufficiently sensitive to record a small amount of tension. More recently de Boer(6) has shown action currents in the frog's heart treated with water at a time when it no longer registered contractions. He does not accept this as evidence of dissociation, however, as the swelling of the heart had caused a shortening to the systolic length and contraction could not be visible on account of the shortening already present. This, in effect, is an admission that there was tension which was not recorded.

The present investigation, undertaken at the suggestion of Prof. A. V. Hill, is concerned with the possibility of separating the production

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of heat from that of tension. The observation on the apparent dissociation by water of the power of a muscle to conduct from its power to contract, suggested one method of experimentation. Another suggestion was found in some data in a paper by Weizsäcker (7) indicating that when a muscle is treated with a dilute solution of alcohol, heat may be evolved at a time when tension is no longer developed.

Methods. The experiments were carried on independently by the two observers. The methods have been previously described (8). A double sartorius preparation was mounted on the thermopile designed by A. V. Hill and Hartree. The thermopile chamber was immersed in water held in a Dewar flask and stirred by a gentle current of air. The muscle was suspended from a spring tension-lever of the type described by A. V. Hill and Hartree (8, p. 115). A watch spring of suitable size was soldered to the end of a U-shaped brass rod. In the middle of the spring was fixed a short lever arm for attachment to the muscle. The advantage of this lever for these experiments is threefold: it is frictionless when used optically as there are no bearing surfaces, it has a sufficient frequency to record without fling, and at the same time is sufficiently sensitive to detect small amounts of tension. The muscles were stimulated through two platinum wires, one at each end. In most of the experiments a short tetanus was used, the duration being controlled by the Lucas gramophone-motor contact-breaker. As galvanometers of different types were used they will be mentioned in connection with the experiments for which they were employed.

The experiments were conducted along two lines. In the first series (Group 1) the tension lever was selected of such a stiffness that the course of the change in the two functions could be followed. In the later series (Groups 2 and 3) a more sensitive lever and galvanometer were used to follow the tension as close as possible to the point of extinction. Both types of data are necessary. It is obvious that whether heat or tension will disappear first will depend upon the relative sensitivity of the galvanometer-thermopile circuit and the tension-lever, and therefore the interpretation must be guided by the course of the heat-tension curve.

The data are presented in Figs. 1 and 2, which show the course of the decrease in a number of experiments, and in Table I, which records the initial and final readings of the heat and tension. The final readings are taken only from experiments in which the values immediately antecedent are known.

Group 1. The first three experiments in Table I were performed by

Hartree using a Paschen galvanometer (8, p. 85) the sensitivity of which was adjusted so that 1 mm. of deflection was produced by 1×10^{-9} amperes. The galvanometer is very quiet and can be read to 0·2 mm., which would correspond to a rise in temperature of the muscle of about 3×10^{-6} degrees C. The tension was recorded by a stiff spring lever carrying a very light pointer to write upon a smoked surface. One millimetre of movement recorded 400 mgm. of tension and, as it could be read to 0·1 mm., it was sensitive to 40 mgm. When the lever no longer moved, presence of tension could still be detected by observing the suspending thread. This is recorded in the table as a "trace." The attachment of the muscle to the lever was such that the figures for tension are twenty-nine times the actual shortening in millimetres. The muscles were stimulated with a 90 ~ alternating current as previously described (8, p. 97). The data record the later stages in alcohol narcosis. They are plotted in Fig. 1 and show quite clearly that a decrease in heat production is associated with a corresponding decrease in tension.

TABLE I.

	Normal tension. mm.	Final tension. mm.	Normal heat. mm.	Final heat. mm.	Final tension. % of normal	Final heat. % of normal	Remarks
1	28·3	Trace	700	3	<0·3	0·43	In air after 5 % alcohol. Not corrected for heat of stimulation.
2	16·5	1·0	93	9	1·6	9·7	In 4·5 % alcohol.
	16·5	Trace	160	1	<0·6	0·6	In air after 5 % alcohol.
3	31·3	1·2	800	26	3·8	3·2	In air after 4 % alcohol.
4	420	0	19	0+	—	—	In 5 % alcohol.
5	383	2	40·5	0·5	0·5	1·0	In 4 % alcohol.
6	310	0	38	0?	0	[0]	Distilled water. Shortening due to swelling, 30 mm. or 9·7 %.
7	420	0	76	<1	0	<1·3	In Ringer's solution 1 part, water 5 parts. Shortening due to swelling, 44 mm. or 10·4 %. Shortening due to chloroform, an additional 318 mm. or 84·6 %.
8	270	0	38	0	0	0	Ringer's solution 1 part, water 3 parts. Shortening by swelling, 31 mm. or 11·4 %.
	620	0·5	80	0·2±	0·08	0·25	4 % alcohol.
	575	Trace	79	Trace	<0·04	<0·25	Ringer's solution 1 part, water

Group 2. A moving coil galvanometer built by Kipp en Zonen of Delft was used for this group. It has an internal resistance suitable for

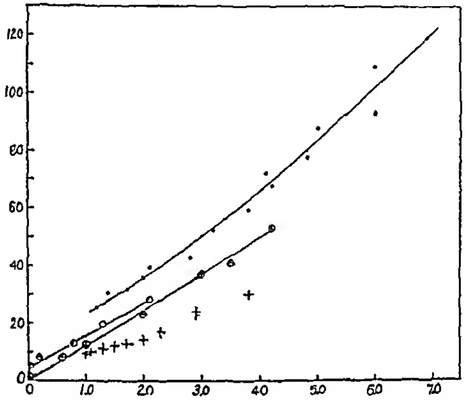

Fig. 1. Effect of alcohol on heat and tension (see Table). Tension abscissa Heat. ordinate. Both in mm. of deflection of the recording instrument.

Exp 1. ⊙ ⊙ Muscle had been in 4½ % and 5 % alcohol Readings made after removing from a final solution of 5 % given for two minutes Maximal tetanus· 0·3 sec Initial heat about 700; tension: 28 3.

Exp 2 + + Muscle in 4 5 % alcohol. Readings made with the muscle in the solution, at intervals between 0 5 and 1 5 hours after immersion. Maximal tetanus. 0 05 sec. Initial tension: 16 5. Initial heat: 93

⊕ ⊕ Same muscle as above. Readings at 2 5 minute intervals after removal from a 10 minute immersion in 5 % alcohol. Normal heat, about 160.

Exp 3 .. Muscle 15 minutes in 4 % alcohol, readings at 3 min. intervals starting 25 minutes after alcohol removed Maximal tetanus· 0 3 sec Initial heat: 800 Tension. 31·3 Curve corrected for heat of stimulating current

use with a 25·5 ω thermopile and gave a quite steady spot. On account of its lower sensitivity stimuli of two seconds' duration were used (1 sec. in Exp. 9). These were maximal induction shocks from a Porter coil. The tension was read by means of an optical lever 3·5 m. long. From a small concave mirror mounted on the lever a beam of light was reflected to a second mirror, and then back to a transparent scale mounted beside the scale, for the galvanometer spot, so that the operator could control the preparation and at the same time read the two instruments. The edge of a card was focussed on the screen and a movement of the shadow

of 0·2 mm. could be detected easily. The lever spring was fairly stiff. As the connections were ordinarily made, the shortening of the normal muscle was in the neighbourhood of 1 mm., corresponding to 550 mm. on the screen. The tension could be read to 35 mg. or less.

The results appear in Table I (Exps. 4 to 10 inclusive) and in Fig. 2. In the recording of the final heat 0? means that there was no visible deflection of the galvanometer but that the spot itself was not sufficiently quiet to allow certainty. The symbol [0] means that the final heat was approximately equal to zero. It is used when there was a small galvanometer deflection at the time of cessation of tension and after killing the muscle with chloroform it was still present in practically equivalent amounts, showing that it was produced by the stimulating current.

The alcohol experiments are in accord with those of Group 1, and distilled water acts similarly, there being no constant differences in the manner by which the two agents produce a decrease of tension and heat. Early readings were not obtainable, due to the unsteady state of the thermopile after the solutions were changed. Concentrations of alcohol which would finally destroy both activities often acted so rapidly that only the final effects could be obtained. About fifteen to twenty minutes were necessary for the galvanometer spot to become sufficiently quiet to allow accurate readings. When the solutions are changed the two sets of junctions must come to equilibrium, and further some heat of solution must be elaborated in the muscle after diffusion of the alcohol. Weizsäcker observed an increase in heat production soon after immersion in alcoholic solution. This appeared in this group of experiments in but one instance eleven minutes after immersion in 3½ p.c. alcohol (Exp. 9). The tension usually began to fall promptly in 3 p.c. to 4 p.c. alcohol, a fact indicating that increased heat production was not often present during the period in which the galvanometer could not be read.

The rate of stimulation being 42 per sec., successive stimuli would probably fall in the relative refractory phases of the preceding (Adrian (9) for values). In this event the second disturbance set up would be subnormal in size (Keith Lucas (10)) and still less capable of passing through muscle conducting with a decrement, particularly if the relative refractory-phase is lengthened in a decrement. It is therefore conceivable that a single induction shock might produce a relatively greater amount of heat in a narcotized muscle, as compared with normal, than a tetanus, because in the latter case any increased heat production by the first shock would be compensated by the relatively greater ineffectiveness of the succeeding shocks. This possibility was tested in two experiments

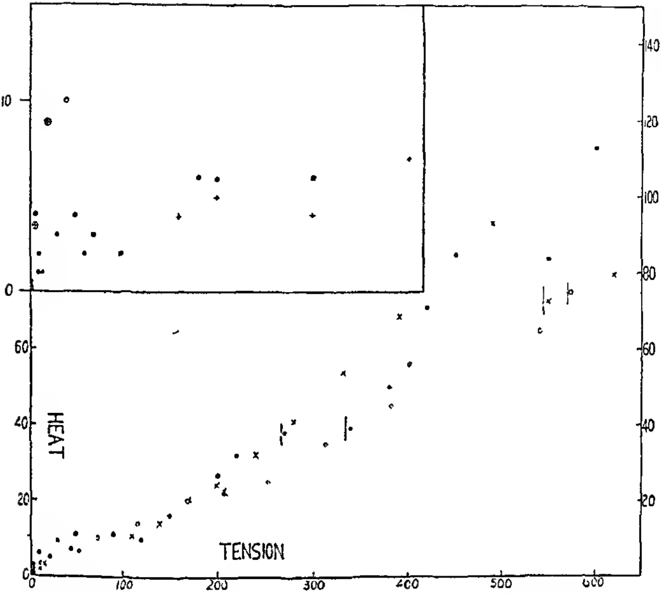

Fig. 2. Effect of alcohol and hypotonic solutions on heat and tension (see Table). Tension: abscissa. Heat: ordinate.

Inset: same abscissæ; ordinates on the larger scale. Main curve made with Kipp galvanometer. Vertical lines after the last reading in Ringer's solution.

Exp. 8. ■ ■

Exp. 9. × × Alcohol 3 %, later 4 %.

○ ○ Same muscle, after recovery from alcohol, treated with Ringer's solution 1 part, distilled water 3 parts.

Exp. 10. ● ●

Inset: data obtained with the Paschen galvanometer and a very sensitive tension lever. Final readings of the series only. The short duration of stimulation, 0·1 sec., decreases the accuracy of the tension readings.

Exp. 11. + + 3 % then 4 % alcohol.

○ ○ Ringer's solution 1 part, water 4 parts, after complete recovery from alcohol. ⊕ ⊕ distilled water after recovery from hypotonic Ringer's solution.

Exp. 12. ■ ■ Exp. 13. ● ●.

potential change, the present trend of the evidence is in the direction of showing that poisons destroy in muscle the process which is responsible for the manifestations, heat, tension, conduction, and potential change. It is safe to conclude that heat and tension never have been dissociated, and it seems likely that they cannot be. Their decrease is roughly proportional. Taking all the experiments into account there is a tendency toward deviation in the direction of inefficiency in the later stages of injury, that is, there is a greater decrease of tension than heat. This difference is not sustained until the final readings and does not affect the general conclusion. It is not intended that the inference should be drawn that H/T must be constant as it is known to vary in different conditions (Hartree and A. V. Hill(13)).

Summary.

When a muscle is narcotised with alcohol or injured with hypotonic solutions, the heat produced and the tension developed on direct stimulation undergo a decrease together and go simultaneously to extinction.

We wish to thank Prof. A. V. Hill for his advice and for extending to one of us the facilities of his laboratory at University College and for instruction in the technique of their use.

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NOTES ON TEMPERATURE AFTER SPINAL TRANSECTION, WITH SOME OBSERVATIONS ON SHIVERING. By C. S. SHERRINGTON.

(*From the Physiological Laboratory, Oxford.*)

THE following observations on impairment of temperature regulation in the paraplegic dog have been obtained incidentally during work which required the keeping of such animals for periods relatively long. The severance of the spinal cord has been in every case performed under deep anæsthesia and with full precautions against sepsis. The animals were housed in ample well-strawed stalls in a warmed room; their general health remained excellent. In some of them the spinal transection was situate as far forward as the cervical region. Since opportunity of this kind occurs infrequently, the observations, although desultory, may possess interest as extending to post-operation periods somewhat longer than are usual.

The impairment of thermotaxis remains severe long after complete subsidence of the acute symptoms of "spinal" shock. A normal dog lightly anæsthetised and immersed up to its middle in ice-cold water for 10 minutes showed a fall of mouth temperature from 37·6° C. to 37·2° C. A small dog of approximately similar weight similarly immersed 530 days after spinal transection at 1st thoracic level, showed a fall of mouth temperature from 37·9° C. (taken in its stall at 27·5° C.) to 33·9° C. Again, similar immersion of an unanæsthetised normal dog of 4 kilos weight brought the mouth-temperature from 37·9° C. down to 37·2° C. in 18 mins.; while similar immersion of this same dog 30 days after spinal transection at 6th thoracic segment brought the mouth temperature from 37·5° C. down to 35·8° C. in 18 mins.

Gardiner and Pembrey have pointed out how clearly the degree of impairment of thermotaxis in paraplegia is proportioned to the extent of the paralysis, *i.e.* is greater the further forward the seat of the spinal severance (cf. also (11)). Observation upon dogs with spinal severance at levels varying between 7th cervical and 3rd lumbar, and at periods subsequent to the transection varying from 21 days to 589 days, exemplifies this proportionality. Thus, Chart 1, three dogs, *A*, *B* and *C*, of

So also in another dog, *L*, with transection similarly between 2nd and 3rd lumbar levels and of 92 days' standing. It (Chart 2 B), with two other dogs, *M* and *P*, with severance between 7th and 8th cervical segments and at 6th thoracic segment respectively, were transferred from stall temperature of 29·5° C. to the cold room at 2·2° C., the room temperature rising to 3·8° C. by the end of the experiment. The temperatures

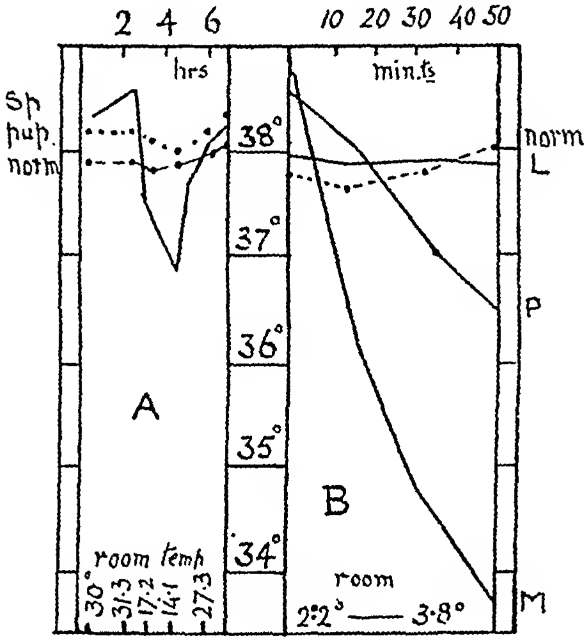

Chart 2.

(vaginal) of *M* and *P* sank to 33·6° C. and 36·5° C. respectively at the end of 50 mins. in the cold room. But the temperature of *L*, although when in the stall somewhat lower than those of *M* and *P*, showed practically no fall from the cold exposure. A normal dog subjected to the test along with the above three spinal, showed slight rise of temperature during and at end of experiment.

Mere abruptness of the change from warm to cold surroundings does not seem to have been a factor of importance in overstraining the paretic thermotaxis. The temperature in dogs with forward thoracic transection sank also when the lowering of the surrounding temperature was gradual and much less extreme. Thus (Chart 2 A) progressive fall of the stall temperature to 14·1° C. during the course of two hours lowered the vaginal temperature of a dog with spinal transection at 2nd thoracic segment by 1·4° C., and caused shivering headward of the lesion. A

normal dog and puppy observed along with the spinal animal showed no shivering, although a slight fall of vaginal temperature.

All the dogs were stalled in a well-warmed room. In early summer the diurnal course of the stall temperature ranged over about 2° C. The temperature (vaginal) of the spinal dogs in this room was noticed to vary more than did that of the normal dogs. The diurnal course of the room temperature and of two of the spinal dogs (i) and (ii) and one of the normal dogs living in the room was followed through a 24-hour period. In (i) the transection lay at 1st thoracic segment and was of 72 days' standing, in dog (ii) at 7th thoracic segment and of 143 days' standing. The observations (Chart 3) showed greater fluctuations of temperature in both "spinal" dogs than in the normal. The fluctuations in the former reflected the oscillations in the room temperature fairly fully, whereas in the normal dog they did so to less extent and less faithfully.

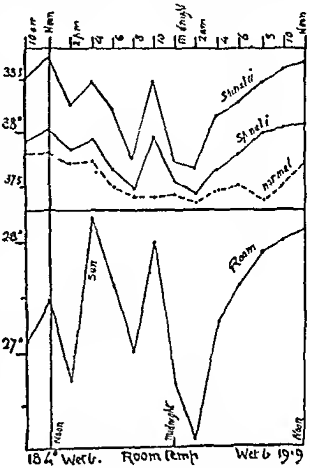

Chart 3.

It was often noted that with stall temperatures of above 27° C. the temperature of the dogs with transection forward in or headward of the thoracic region was higher than that of the normal dogs; with stall temperatures below 23° C. the temperatures of the latter were above those of the former. But spinal dogs with transection even as far headward as 7th cervical level, quartered for long periods, e.g. in individual cases upward of 18 months, in stall temperatures ranging between 25° C. and 30° C. remained in excellent general health and nutrition, though at times their deep temperatures must have undergone considerable fluctuations. Even the "cervical spinal" dog retains in certain measure an effective thermotaxis. Thus, such a dog removed from a stall temperature of 28·6° C., with vaginal temperature 38·1° C., to a room at 19·8° C., where it soon began to shiver in head and neck and continued to do so, showed, when after 60 mins. its temperature had fallen to 37° C., a further fall of only ·2° C. at the end of a further hour and a half. No ill-effects accrued

from the exposures made for observations in the cold room. The longest exposure there was 60 mins. with the room between 1° C. and 2° C. The animal, with transection at 8th cervical level, showed then a vaginal temperature of 32·3° C., and its respiration was 14 per min. Returned to its warm stall (28° C.), its temperature there rose and reached 37·5° C. in 56 mins.

With the cold room two points observed were the following. In the two dogs *K* and *L* with transection between 2nd and 3rd lumbar segments the hind feet grew cold in the cold room seemingly as rapidly and fully as did those of the normal dogs: the shivering, however, never encroached on the hind limbs further than the groin (? psoas muscle). In one animal with transection at 2nd thoracic level, though the feet remained "warm" in the cold room, the pinnæ-tips certainly cooled somewhat. In other dogs with transections at 2nd and 1st thoracic or 8th or 7th cervical levels the pinnæ remained "warm" as did the feet.

As to cold shivering in the paraplegic dog, Goltz, from observations by Freusberg, considered the shivering to be spinal. Richet (32), however, noting that in the chloralised dog severance of the spinal cord at once confined the field of shivering to muscles headward of the spinal transection, concluded that shivering requires a prespinal nervous mechanism. It remained open whether cold shivering as a spinal reflex might not become possible when sufficient period had elapsed for recovery from the depression of reflex action which ensues for a time on the transection. In regard to this, Laslett and myself (38) showed that the absence of cold shivering from the paraplegic region still persisted in dogs after complete subsidence of all depression from the spinal shock following transection. Thus, we instanced a dog retaining excellent general health for 580 days after exsection of the 8th cervical spinal segment, in which free shivering, when the dog was cooled, occurred headward of the lesion but no trace of shivering ever trespassed backward to the muscles innervated from behind the lesion. So similarly in dogs 300 days after exsection of 2nd thoracic segment and 315 days after exsection of 5th thoracic. Observations of this kind repeatedly made in other chronic transection experiments have always given me the same negative result. Shivering was absent from the paraplegic region as completely a year and a half after spinal severance as at first, and was so although the usual spinal limb and other reflexes, cutaneous and deep, were brisk, and the femoral pressure (Hill's sphygmomanometer) as high as about 122 mm. Hg. Shivering was absent, although by the kymograph and open artery method fair vaso-constrictor reflexes were

obtainable, under stimulation of the skin and various afferent nerves of the paraplegic region. Recently Barbour and Tolstoi in a dog surviving seven days after spinal transection between 6th and 7th cervical segments point out the absence of shivering behind the lesion when cooled to rectal temperature 32·6° C. And Rogers and Lackey report an observation that in a dog with mid-thoracic transection exposed to cold nine months after transection the hind quarters did not shiver.

The non-appearance of cold shivering in the paraplegic region of the dog can reveal with great clearness the exact segmental level of the spinal lesion by demarcating the muscle-field innervated behind the lesion from that in front. The boundary so shown will actually subdivide an individual muscle, *e.g.* the anterior part of deltoid will shiver violently while in its posterior part no shivering can be evoked. In paraplegic man also (Kennaway and Pembrey) shivering fails below the lesion, and could exhibit the segmental level of a traumatic lesion.

Returning to the possibility of eliciting cold-shivering as a spinal reflex, direct observation as mentioned above revealed that the surface of the skin innervated from behind the spinal lesion, instead of turning cold in the full normal way under exposure to cold, continued fairly warm. There remained the possibility therefore that a stimulus, *i.e.* coldness of skin, which might be requisite for shivering as a spinal reflex, was in the above experiments locally wanting. To escape this possible source of negative result, spinal dogs carefully supported by the axillæ were immersed in ice-cold water nearly up to the front limit of the paraplegic region. How fully they had recovered from spinal shock is shown by the immunity with which even those with cervical transection bore this nearly vertical "head-up" posture, demonstrated by L. Hill to involve fainting where severe vasomotor paresis is present. Such posture is easily fatal to a dog in the first month after cervical transection, and three animals were lost early after operation in this way while being carried by an unskilled attendant. The immersion was continued until the immersed skin felt quite cold, indeed remained so long after withdrawal from the water; yet no trace of shivering set in behind the transection. The experiment was repeated on a number of "spinal" dogs at various times; always with failure to produce shivering behind the lesion. The ice-cold water cooled the skin throughout the spinal regions severely. The conclusion drawn is that shivering to cold cannot be produced as a spinal reflex.

It was frequently noticed that in the paraplegic hind limbs after immersion in ice-cold water, *e.g.* for 15 mins., the ordinary reflexes,

ipsilateral flexion and contralateral extension could with difficulty be evoked, at least from the skin. That this was due to merely peripheral numbing is probable from the observation by Sturm van Leeuven and van der Made that central cooling of the decapitated cat, even to 30° C., does not much depress these reflexes. Moreover in my experiments the knee-jerk remained readily elicitable though greatly altered in character, having, as Britton has shown, become of extreme tonic type. The slight active extension of the knee produced by each jerk relaxed so slowly that the next jerk even 2 secs. later found the knee still in approximately the same degree of extension to which the previous jerk has brought it. Britton notes that exaggerated tonic spasms of the postural musculature occur when the temperature of the recovering animal stands between 21° C. and 25° C.

There was, however, in the paraplegic dogs acute shivering headward of the lesion, and the mouth and rectal temperatures fell rapidly, the latter on one occasion to 32·2° C. although the thermometer was inserted very far. No ill-effects accrued from any of the exposures to cold by immersion; Britton has shown that the cat recovers after cooling to a rectal temperature as low as 19° C.

As to the shivering in the non-paraplegic region, it was frequently seen to occur when (*vide supra*), after transection between the 7th cervical and 2nd thoracic levels, the dog's ears, nose and feet felt warm.

In a cold pail experiment, shivering was observed in the head and neck in a "cervical spinal" dog when with a room temperature of 24° C. the pinna temperature as read by surface thermometers was 34·3° C.; the mouth temperature reading 36·3° C. The ears of the normal dog plunged up to its middle in the ice-pail soon felt quite cold as compared with those of the cervical paraplegic dog.

Observations were then carried out on the non-paraplegic, so to say "normal," region of the dog by immersion of the paraplegic region in ice-cold water in a heated room. The pail of iced water to receive the insentient portion of the dog was set in a waterproof sack; directly the immersion was begun, the mouth of the dry rubber sack was drawn round the chest close below the headward limit of the skin anæsthesia. An attendant supporting the dog kept it quiet and amused. The room temperatures varied on occasion between 30° C. and 60° C. and finally 63° C. An additional thermometer was suspended close to the dog itself. The following is a sample of results.

White smooth-coated terrier: transection at 8th cervical 19 months previous. At 3.42 p.m. in stall at 27·5° C., vaginal temperature 38·3° C., mouth inside cheek 38·1° C.,

pinnæ feel quite warm, feet also Respiration 27 per min At 3 58 p m removed to hot room Dry bulb 61° C ; wet bulb 31° C Immersion commenced At end of 3 mins pinnæ and fore feet feel as "warm" as before, but there seems to be slight shivering At end of 4th minute obvious shivering in temporal muscles, pinnæ and fore feet feel quite "warm" At end of 5th minute shivering greater and in neck as well as head, pinnæ and foot still feel quite warm. At end of 6th minute, shivering more Ear and fore paw feel 'hot and dry." Resp 24 per min Mouth inside cheek 37 8° C Dog taken from water and dried Vaginal temperature 37 3° C. rapidly rising in the hot room to 40° C The shivering had therefore occurred when, although the blood temperature had fallen somewhat, the sentient skin itself seemed still warm

A possible reflex source for the shivering might be the lung, though this is unlikely considering the warmth of the air breathed in the just-mentioned experiment. A smooth-coated terrier, 1st thoracic segment exsected 11 months previously, was immersed in ice-water as described above, but in a room of temperature 66° C. to 67·5° C During the heating of the room previous to the observations the wet-bulb thermometer stood at nearly 31° C. when the dry bulb was close on 60° C. Four days before the immersion experiment the right vagus was severed in the neck with full anæsthesia. On the forenoon of the day of immersion the left vagus was similarly severed In the afternoon, an hour prior to the immersion, a small opening was made just below the xiphoid, *i e* much behind the front limit of body anæsthesia. In the stall at 27·7° C. the temperature given by a thermometer inserted against the liver was 38·8° C. The thermometer was re-inserted and another, an "Immisch," placed with it, short threads from them issuing at the opening The bulb of a long-stemmed thermometer was also put through the xiphoid opening so as to lie to left of mid line between liver and diaphragm where the ventricles of the heart in the "head-up" position of the animal rest on the latter. The thermometer stem outside the xiphoid opening was for most of its length covered with thick cork. Removed to the hot room, the anæsthetic body of the animal was then gently placed in the pail of water, but not so deeply as usual: the mouth of the dry rubber sack in which the pail was set was drawn round the animal's girth below the thermometer; in 1 min. the thermometer reading was 39·1° C, and 3 mins. later, the immersion, etc., having been then fully adjusted, was 40 6° C. Half-a-minute later, the thermometer, having reached 40 8° C., ceased to show further rise. About 20 secs later the thermometer reading was noted to be falling, the ears and fore-feet felt "hot," and the animal was panting acutely. The mucosa in the mouth was not dusky but pink The thermometer continued to fall gradually; panting ceased entirely when it reached 38·4° C., but the ears and skin of head and fore paws still felt "hot." Shivering commenced 13 mins 20 secs. after commence-

ment of the immersion, when the liver thermometer marked 37·1° C. The ears and head and fore-feet still felt "quite warm." One of the short thermometers partly withdrawn read 37·2° C. The shivering began in the temporal and lower neck muscles almost synchronously. During the next 2 mins. the shivering increased, never however extending behind the transection level, and the liver thermometer fell to 36·8° C. The ears and head and fore-feet still felt quite warm. The immersion, which had lasted 15½ mins. was then discontinued. The clinical thermometer was then withdrawn through the xiphoid opening and marked 41° C. as the maximum it had reached. The observation illustrates that heat-panting, as Richet (31) showed, and that also cold-shivering, can take place after double vagotomy. As to the cold-shivering, the skin-surface headward of the spinal transection and therefore still through afferent nerves in normal rapport with the prespinal centres had in contact with it air at above 60° C. Moreover the sentient skin-surface was not cooled by sweating; no sweating was observed and the transection presumably excluded it. There was certainly none in the paws, from which observation had also on many previous occasions consistently shown it to be absent. Yet when, as read by the liver thermometer, the deep temperature sank to 37·1, shivering began. The *surface temperature* of the sentient skin could under these circumstances hardly have been below the blood temperature, *i.e.* 37·1° C. But that figure is a fairly high surface temperature. If cold-shivering be provocable through afferent nerves of the skin, those nerves are presumably the afferent nerves from the cold-spots, whose receptive organs lie probably at junction of cutis vera and epidermis, the latter about 1 mm. thick. Their temperature in this experiment when shivering began was presumably between that of the blood 37·1° C. and of the air at 67° C. separated from them only by the epidermis perhaps 1 mm. thick.

Considering that the cold-spots presumably often lie at a temperature below 37·1° C. and yet no shivering occurs, a cold-spot temperature above 37·1° C. can hardly *per se*, if the adequate cold stimulus be temperature distance below normal skin warmth, constitute a shiver-stimulus. In the above experiment the shivering set in, it is true, during somewhat rapid fall of the blood temperature and when the skin temperature, despite the hot air acting on it, must therefore have been sinking. Yet, admitting a "physiological zero" (on Hering's view of temperature sensation) possibly higher than usual, the fall from it hardly relieves the improbability that in this experiment the fall was a shiver-stimulus to the cold spots, their temperature being of the height it was. The experi-

ment seems difficult of explanation on the reflex view if the peripheral stimulus for shivering consist in subnormal temperature of the superficial skin, or in a sufficient fall of superficial skin temperature from an adaptation zero. But on the view of the nature of the adequate stimulus for the "cold" spots arrived at by Ebbecke in his interesting paper, the experiment seems compatible with the observed shivering being reflex. Ebbecke supplies evidence that cold sensation originates when warm blood flows through cold skin or when relatively cold blood flows through warm skin. With this latter condition the experiment markedly complies. Adopting Ebbecke's view of the "cold" skin-stimulus, the issue of the experiment seems to narrow itself to the shivering being either a reflex evoked from the skin, or a reaction of deep source reflex or "central."

A source "deep" in situation but still reflex in operation, might be for instance in the receptor organs of skeletal muscle. Examination of this possibility was attempted by severing afferent spinal roots. In a small smooth-coated terrier five consecutive afferent roots for the right hind limb were severed, under full anæsthesia; two months later the animal, otherwise normal, was partially immersed in cold water. In less than 2 mins. shivering set in, and soon involved the muscles of both hind limbs, the right somewhat less strongly than the left. Similarly with a rough-coated larger dog with seven consecutive afferent roots severed. In another with severance of five afferent roots to right fore limb the muscles of the right limb shivered, but very distinctly less than those of the left; the normal limb shivered sometimes without the deafferented. [illegible]ivers in the deafferented limb were noted to be always synchronous [illegible]ose of the normal. Bazett and Penfield noted bilateral [illegible] after unilateral decerebration in the cat. The observations [illegible]at muscles, when no longer in afferent connection with the [illegible]entral nervous system, still exhibit shivering from cold.

Taken together, the observations tend, so far as they go, to refer the shivering to either a cutaneous reflex, the mode of stimulation propounded by Ebbecke explaining its excitation, or to a "central" origin, *e.g.* a direct effect of temperature on the central nervous mechanism. This latter was the conclusion come to by Richet so far back as 1892, and is an alternative strengthened by analogy with the evidence on thermal polynœa (Richet (31)), and the results established by Barbour (1) and his subsequent co-workers (3, 4, 5), also by Hashimoto, from experiments with localised cooling and warming of the brain; and by observations of O'Connor and of Heymans on cooling and warming the blood supplying the head. In regard to the "central" origination of

shivering, one might perhaps hardly expect that it would cover cases where shivering is an adjunct, as experience shows, not only to one and the same particular blood temperature, but to temperatures varying with varying circumstances. Richet accepts, and so similarly, if I understand them aright, do Barbour, Heymans, Rogers and others, the probability of the occurrence of reflex shivering as well as "central." The experiments reported here seem conclusive against *spinal* shivering, but seem quite compatible, by adoption of Ebbecke's view of the stimulation of skin by cold, with shivering as a prespinal reflex.

The *locus* of the central nervous mechanism of cold shivering, either reflexly or centrally stimulated, is clearly prespinal. The shivering being an item of general thermotaxis (Richet (33, 34), Pembrey (28, 29), Johannson), its central localisation may correspond with that for general nervous temperature-control. This in the pigeon is shown by Rogers (35, 36, 37) and co-workers to be thalamencephalic. Bazett and Penfield, on decerebrate cats, *preserved in one case for as long as 26 days, with very* complete temperature records, obtained no shivering, and point out that their experiments in conjunction with those of De Barenne on the cat kept five months with total brain-ablation above the thalami, show that control of temperature in the cat is "definitely located between the level of the middle of the superior colliculus and 2 mm. in front of pons and the upper limit of thalamus." Rogers and Lackey write that after destruction of the thalami (pigeon) there is loss of response "to atmospheric cold by increased activity of the striated muscles such as shivering." The supervention of shivering later than spinal activities in the mammal awakening from hibernation (Pembrey and Pitts (30), Pembrey (29)) thus seems natural. Britton finds that in recovery from cooling to 16° C. (rectal) a number of spinal reflexes, *e.g.* knee-jerk, Achilles thrust and stepping return earlier than shivering. In my own experience chloroform depresses and abolishes shivering earlier than it does many spinal reflexes.

Spinal transection impaired, of course, the dogs' adjustment to heat as well as to cold. In dogs with cervical transection it was constantly noted that when they were exposed to heat the tongue at frequent intervals was swept forward to moisten the snout. The noses of these dogs were continually dry. After mid-thoracic section, the hind-feet were constantly dry as well as warm; similarly the fore-feet also after severance at the 8th cervical level. The absence of heat-sweating in the paraplegic region is well known from observation by numerous observers, although profuse

sweating unrelated to heat occurs in the paraplegic region in patients as a spinal reflex (Head and Riddoch). A dog with transection of 132 days at 1st thoracic level transferred from a stall temperature of 26·5° C. where its temperature (vaginal) was 37·9° C. to a hot room at 61° C. showed at end of 25 mins. a temperature of 41·6° C. It began to pant violently about 12 mins. before removal from the room. But its feet though hot remained perfectly dry throughout. The animal did not, apart from panting, seem distressed by the high temperature. A dog with transection at mid-thoracic level similarly exposed showed a rise of temperature to 39·6; its fore paws sweated freely but the hind paws remained dry.

The contrast between normal and paraplegic dogs in their reaction to rise of external temperature was illustrated by the following. Three "cervical" dogs, *R*, *S* and *T*, in good nutritive condition, and of weights about 7, 6·5 and 9 kilos respectively. *R*'s transection at 7th cervical, *S*'s between 8th cervical and 1st thoracic, and *T*'s through the 2nd thoracic, the operations respectively 186, 294 and 148 days prior to the heat exposure. They, along with a normal adult terrier *N* and a 12-weeks puppy, were exposed in their stall to a rising temperature, the temperature finally reaching in somewhat over three hours 48° C. dry-bulb. The wet-bulb temperature, important in this connection (J. S. Haldane (14, 15)), was not observed at the maximum temperature of the room, but when the room was under process of heating its wet-bulb temperature had read 29° for dry-bulb 44°. Dr Haldane draws my attention to the certainty that the wet-bulb temperature would, as the experiment proceeded, have been influenced, not only by the rising room temperature, but by the further wetting of the air from perspiration and respiration. So that the preliminary wet-bulb observation understates the severity of the ultimate exposure. Chart 4 gives the course of the observations. The room temperature was observed in each of the three open stalls, rather widely spaced, occupied by the three paraplegic animals. The wooden floor of the stalls was some 9 ins. above the cement floor. The normal dogs were free in the stall-room. The room was 32 ft. by 22 ft. by 10 ft. and was heated by hot pipes, electric heaters and a gas stove. A hot summer forenoon was chosen. The temperature of *R*'s stall rose rather higher than did *S*'s; conformably *R*'s body temperature (vaginal) rose higher, *i.e.* to 42·4° C. than did *S*'s. Dog *T* had received its bath (in water at 33° C.) about one hour before the raising of the room temperature. It had been carefully rubbed and "dried," but its coat was still moist. Its temperature started lower than that of the two other spinal dogs, nor

did it rise so high as theirs. It was a rather larger animal. The feet of all the spinal dogs remained dry throughout the experiment, though the

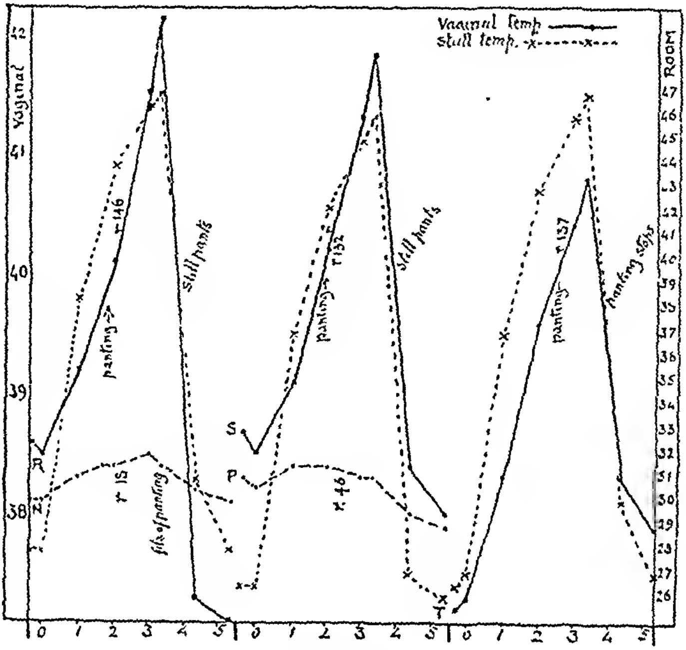

Chart 4.

feet were thought to feel even warmer than usual. Conversely, the feet of the normal dog and puppy sweated so profusely that their steps left dark prints of moisture on the cement floor. The ears of the normal dogs were hotter to feel than those of the spinal dogs though these latter were quite warm. Panting began almost simultaneously in dogs *R* and *S*; *T* did not begin to pant until 20 mins. later, when the temperature of the room was higher. Panting became marked in each case when the temperature (vaginal) lay between 40·2° C. and upon a further rise the panting became violent. Thus in *R* respiration rate 146 per min., in *S* 132, and in *T* 137. At a time when the puppy, with a temperature 38·7°, was breathing 46 per min., and the normal dog, with a temperature 37·8°, was breathing 18 per min., the normal dogs' ears were however hotter to palpation than were those of the spinal dogs, and the normal dogs' feet were wet with sweat. An hour later than spinal dog *T*, and when the room temperature was 45° C., the normal dog began to pant, not continuously but in short fits. Its vaginal temperature was then 37·9° C. Despite their panting and high body temperature, the spinal dogs showed

at no time signs of illness or distress. They watched with evident interest our movements about the room, greeted with eagerness their turn for a temperature taking, and each was impatient of attention received by another. Nor did any of the dogs show ill after-effects. The same was not the case, however, with some other animals in the room. Prior to the room temperature being raised, all animals quartered there, except the five dogs mentioned, had been removed lest they might suffer harm from the temperature which it was intended the room should reach. But I had made an exception of three African monkeys (*Cercopithecus callitrichus*), in the belief that they, from a tropical climate, would like rather than dislike the heat. Small monkeys of about 3·5 kilos each and in excellent condition, they were housed two together and one apart in two cages, each of about 34 cubic feet, having one side open save for thin iron well-spaced vertical bars, and a partly open grid top. The two cages stood on a long piered slate slab 1 ft. 6 ins. above the floor, *i.e.* about 9 ins. higher than the dog stalls. The higher of the two thermometers recording the temperature of each dog-stall hung at above the level of half-way up these cages. After the work of observation on the dogs began we paid no attention to the monkeys. It was when the experiment was at its height and the dogs' and stalls' temperatures had been taken at 3 o'clock that I noticed one of the monkeys prone in its cage. I found on lifting it that its skin felt very hot. It was unconscious and its companion nearly so. The third monkey occupying a separate cage lay also unconscious. All three were taken promptly from their cages and carried quickly to another room where they were plunged and bathed in water. One never regained consciousness; its rectal temperature was 43·1° C. The two others revived with the cool bathing. One of these seemed somewhat delirious during the following three hours. Next day both appeared well, took food and behaved as usual; nor did further ill-effects follow. As for myself and the others who took part in the observations in the hot room, we experienced no real discomfort either then or afterwards, although in it as long as were the animals, and working hard there. One factor determining the quicker rate at which the exposure affected the monkeys would be their relatively smaller size and mass-surface ratio. The great influence of this factor is strikingly shown by Haldane's observations (14, 15), in which, for instance, a saturated-air temperature of 48·5° C. occasioning a rise of human body-temperature of about 2·6° C. in 15 mins. caused a fatal heat-stroke to a canary in 3 mins. "A small warm-blooded animal can be used as a rapid test of a hot atmosphere which will in time be dangerous to man." Haldane, p. 556 (15).

In Chart 4 "panting" indicates where Richet's (30, 33) "polypnæa" appeared. The polypnæa often sets in suddenly; but it can be preceded, and in these experiments was so, by gradual considerable increase of breathing frequency, Heymans' "tachypnæa." The chart marks commencement of full polypnæa only approximately. Bazett and Penfield in their observations with precise automatic temperature registration in cats surviving transcollicular decerebration, point out that though full heat-polypnæa did not develope, the progressive rise of body temperature was accompanied by progressive acceleration of breathing frequency. In agreement with their experience has been my own failure to find heat-exposure produce full thermic polypnæa in the decerebrate dog or cat, though when the rectal temperature has risen to 41° C. the breathing rate may be trebled, and may be accompanied by some accessory respiratory movement of lower jaw and cheeks. But I have not then seen "panting" in the sense of respiratory to-and-fro tongue movement visible through an open mouth and retracted lip-commissures, and breathing rates of 130 and more per min. as in the paraplegic dogs of the above experiment exhibiting true heat-polypnæa. In an observation with Mr Liddell, on a cat decerebrated 4 mm. anterior to the anterior colliculi, exposure for 65 mins. to a room temperature rising from 27° C. to 34° C. raised the rectal temperature from 37·6° C. to 42·7° C. When rectal temperature reached 41·1° C. the breathing frequency which at commencement had been 30 had gradually increased to 75 per min., but at 42·5° rectal temperature the breathing rate was 66 per min. and although slight respiratory action of jaw and mouth angles had developed, the preparation succumbed gradually to the heat exposure, when the rectal temperature reached 42·70° C., without at any time developing "panting" or true thermal polypnæa.

Often with a high vaginal temperature the spinal reflexes in the paraplegic dogs became noticeably brisk. This difference from the temperature effect on the decapitated cat (41) may depend on the circumstance that in these dogs the period of spinal shock had had time to pass off. In the decerebrate cat warmed to a high vaginal temperature, *e.g.* 40°, violent running movements are apt to be brought on by even light handling of the preparation. In traumatic paraplegia the febrile depression of spinal reflexes described clinically by Head and Riddoch would seem therefore, as they suggest, to have its cause in toxæmia rather than in the febrile temperature *per se*.

In their resistance to exposure to heat normal dogs and cats seem to present considerable individual differences even apart from relation

to weight, age, coat and nutritive condition. Thus five normal dogs were placed in a room which was heated to 66° C. prior to observations on a spinal transection animal. They were free, *i.e.* not in stalls. All were small mongrel terriers of 3–4 kilos, in good health, about the same nutritive condition, and, though adult, young. One of them, after some 20 mins., removed itself to a corner where the temperature may have been less, and there lay down. Its behaviour excited no further attention until about 10 mins. later, when it was noted to be panting and salivating. Its temperature (vaginal) before entering the warm room had been 37·3°, and no higher than that of the others. At the end of 37 mins. in the hot room this dog, evidently getting worse, although the others seemed quite comfortable, was removed from the room, very excited, seemingly delirious, tossing its head about, panting too rapidly for a count by inspection, salivating extraordinarily profusely, sweating freely from the feet, with pinnæ which felt hotter than any dog's I had felt, and with nipples distinctly redder than at first they had been; likewise the tongue, oral mucosa and fauces were bright pink. The vaginal temperature taken immediately on removal from the room was 43·2°. In a cool room the temperature subsided quickly and no ill after-effects ensued. The other dogs, remaining in the room some 5 mins. longer, were in no distress from the heat; vaginal temperatures below 39°; occasional short fits of panting never prolonged or violent. The distressed dog was neither the largest nor the smallest of the dogs; it weighed 3·4 kilos, its coat differed from the others somewhat in being slightly "broken," *i.e.* rougher haired, but was of the same colour, white, like the rest. Professor R. S. Woodworth, who kindly took part in the observations on this as on some other occasions, the attendant, and myself felt no malaise though in the hot room longer than the dogs. Professor Woodworth's pulse was 124, mine 121, at end of our work in the hot room.

Again, in a room with 15 cats, all young and of about like size, stalled singly in similar cages in three tiers, the temperature raised from 26° up to 35° in 70 mins. brought no disturbance to any of them but one, a cat in the lowest tier, not at the hotter part of the room. Just at close of the exposure period this cat was seen to fall, then clumsily rise, panting; it seemed slightly delirious, the tail tip was lashed, the claws extended, and the jaws snapped "at the air." Rise of room temperature was at once stopped. The rectal temperature marked 39·5°. The panting continued for a time while the room temperature fell quickly. Forty minutes later the cat seemed quite recovered and received its meal. Three days later the same cat with 14 more, some of them of the previous exposure,

and all now chosen as closely resembling it in age and size and condition, were exposed at the same time of day as before to warming of the room from 26° up to 39° in 70 mins. Again the heat embarrassed no other of the cats than it. Three of them lay curled up quietly asleep throughout; others slept at times; others paced their cages inviting attention. The cat that had shown signs before was this time in a cage of the top tier half way down the room. Its breathing had been 23 per min. at commencement: when 22 mins. had elapsed, it was breathing 108 per min., the room being 32·5°. It was breathing slower, 74 per min. a little later when room temperature was 33·5; its paws felt dry; those sampled in two others were slightly moist. At the end of one hour it was lying down breathing 124 per min., but not panting with open mouth; in the cage next to it a sleeping cat was breathing 12 per min. The breathing of two other cats, then pacing their cages, was 25 and 36 respectively. Ten minutes later, when the exposure finished, the cat was breathing less quickly, 119 per min., though still lying down at full length and obviously distressed, yet not actually panting. Its rectal temperature then taken was 39·2°; that sampled from two of the others 37·9° and 38·1° respectively. The cat that showed distress was 2·6 kilos, healthy, and neither fat nor lean, not the largest nor the smallest of the batch, and, like the rest, scarcely yet full-grown.

Summary of Conclusions

In dogs long after complete subsidence of spinal shock as judged from usual post-transection reflexes, there persists in the region innervated from behind the transection marked failure of adjustment of the surface blood-supply (paws, pinnæ, nose) to changes of surrounding cold and warmth. The failure of this vascular adjustment, though not absolute, remains severe and without improvement; along with it there is complete abeyance of sweating to heat and in the muscles complete abeyance of shivering to cold. Diurnal fluctuation of 2° C. in the stall temperature affected conspicuously the vaginal temperature of cervical paraplegic dogs fully recovered from spinal shock.

In dogs all attempts, even by considerable exposure, to induce cold-shivering in the paraplegic region as a spinal reflex entirely failed.

On cold immersion of the insentient paraplegic portion of the dog, shivering occurred headward of the spinal lesion even when the skin surface headward of the lesion still felt to the touch fully "warm." Shivering under these circumstances is, on older views of the nature of the adequate skin-stimulus for "cold," difficult to explain as reflex,

but is yet not precluded from being reflex on the view propounded by Ebbecke for the nature of the adequate skin-stimulus for "cold" sensation. Shivering under these circumstances may, on the other hand, be possibly of deep origin from direct cooling of a central (diencephalic) central thermotaxic mechanism.

Shivering seems not to require the afferent nerves of the lung or of the shivering muscles. Shivering, reflex or "central," requires, like Richet's heat-polypnœa, some central nervous mechanism anterior to the mid-brain.

Instances where, under severe but short-lasting changes of surrounding temperature, the body temperature (vaginal) of the dogs fell to 32·3° C., or, on the other hand, rose to 42·5° C., were not productive of serious mischief, and after the animal's return to its stall at 27–28° C., the body temperature quickly regained its approximate normal. A rise of temperature (vaginal) reaching 43·2° C., productive of delirium, profuse salivation and acute symptoms of distress, was yet rapidly recovered from on removal to cool surroundings. It would seem therefore that even in high paraplegia, thermometry of somewhat frequent interval can, as Pembrey has pointed out, notify over-stress of thermotaxis in ample time for the taking of successful measures of relief.

I tender my hearty thanks to Dr Haldane and to Professor Pembrey for valued counsel from them regarding the accompanying statement of these admittedly desultory observations.

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THE RELATIVE INFLUENCE OF MENTAL AND MUSCULAR WORK ON THE PULSE-RATE AND BLOOD-PRESSURE. By R. D. GILLESPIE, *McCunn Scholar in Physiology.*

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Studies of the changes in pulse-rate resulting from various kinds of stimuli are numerous. As Tigerstedt has remarked: "It is an old and well-known fact, that the pulse-rate changes in one or other direction under the influence of all kinds of stimuli." But not much is known of the influence of mental work on the rate of the heart, and in the results obtained there is a lack of unanimity. Yet the question is of some importance; for it has been suggested by Benedict(1) that "pulse-rate indicates, in a general way, internal muscular work and muscular tonus" and is consequently within limits, an index of metabolism. For this and other reasons it is desirable that definite data should exist as to the effect of mental work on the rate of the heart, and also on the blood-pressure. The data so far obtained are as follows:

Pulse-rate. Vaschide(2) observed a diminution in his pulse-rate during a period of nine days in which he worked for an examination, as compared with a period when he did a similar amount of light work (attending classes, etc.) but little or no mental work.

Benedict and Carpenter(1) found a rise in pulse-rate in students working at an examination paper. Dodge(3) criticised their methods, on the ground of unsatisfactory controls, but confirmed their results. Dodge's own methods are open to criticism. He investigated the pulse-rate changes in only three subjects, and this at a time when they were under a certain amount of emotional stress. In investigations of this sort it is essential to eliminate so far as possible all emotional factors. Dodge, however, recognised the importance of obtaining a basal rate before commencing an experiment, and attributed the lack of unanimity in other observer's results to neglect of this.

According to Binet and Henri(4), mental calculation lasting for periods varying from a few seconds up to 3 or 4 minutes has almost constantly the effect of accelerating the pulse by 5 to 20 beats per minute. They attribute the acceleration to nervous influences. Mosso(5)

found, in observations on persons with cranial openings, that mental work was accompanied by an increase in brain-volume and an increase in amplitude of the cerebral pulse-wave. A plethysmographic tracing taken simultaneously from the forearm showed, however, no change. Hence the alteration in the cerebral pulse is, according to Mosso, not due to increased heart-action. Gley (6) confirmed this, and attributed the result to vasomotor influences on the carotid. Morselli (7) stated that the increase in brain-volume did not occur immediately and considered that it was caused by psychic activity. Binet and Courtier (8) experimented with prolonged intellectual work, and noted that the pulse was small and dicrotism high. Ioteyko (9) concluded that a strong short intellectual effort produced vaso-constriction and acceleration of pulse, followed by a slight falling off.

Blood-pressure. Kiesow (10) obtained negative results, while Binet and Vaschide (11) observed an increase of blood-pressure in the hands. Lahy (12), measuring the blood-pressure just after the cessation of mental work, found that it was greater than the blood-pressure existing before the experiment began; and on comparing the results obtained by measuring the blood-pressure of soldiers after a march, with that of typists after a fast typing exercise, concluded the blood-pressure rose higher in mental than in physical work. This rather astonishing result depends on two fallacious assumptions: (*a*) that measurements taken after work are a reliable index of the changes in blood-pressure during work, and (*b*) that the muscular movements of typing produce no change in blood-pressure. L. Hill (13) observed an increase in blood-pressure as the result of what he cautiously calls "mental excitement"—the result of his ordinary day's work of lecturing, etc.—as compared with his blood-pressure on a holiday. Pillsbury and Griffiths (14) found a decrease in blood-pressure after mental work; but their observations were made only after work, the differences recorded were very small, and they were not always careful to ensure that a resting-level had been attained before work was begun.

Methods. The mental work in our experiments consisted as a rule in the addition of vertical columns of 4 single-digit figures. The figures were arranged in the way suggested by McQueen (15), only the numbers from 5 to 9 being used, so that the 4-figure additions were of fairly uniform difficulty. The band of white paper on which the figures were inscribed (in blue, in large script about 5 cm. high and easily legible at the required distance of nearly 2 metres) travelled over three drums, one of which was actuated by an electric motor. The speed of presentation of the figures could be varied to suit each individual. The figures moved from right to

left, the subject adding vertically and proceeding from left to right. A screen was arranged so that no more than six columns of figures were exposed at the same time. This allowed for variations in the rate of addition, and facilitated the subject's attention by eliminating the rest of ths figures from his vision. The experimenter noted the results of addition as they were called out by the subject, at the same time marking the passage of 10 sec. intervals: the rate of addition could thus be estimated. Twenty-three subjects were available and over 80 experiments in three series were carried out.

The blood-pressure readings were taken by means of a carefully-calibrated Tycos instrument attached to the arm placed at the level of the subject's heart. A Tycos was used because from it readings can be taken rapidly with a minimum of disturbance to the subject. The pulse-rate was counted at the wrist. In all cases, care was taken that the blood-pressure and pulse-rate had reached resting-level before an experiment. The duration of an experiment varied from 5 to 30 minutes. Three distinct series of experiments were carried out—the first series with a group of ten male students, the second with fivs (male) laboratory workers, and the third with a group of eight women students. The second series was especially directed to the elimination of emotional and other factors which might invalidate the results of the entirs research.

Series I. In the first series of ten male students, a comparison of the blood-pressure and pulse-rate changes occurring in mental work was made with those occurring in the same subjects in muscular work, and in simultaneous mental and muscular work. The muscular work consisted in exercise with one arm on the convertible ergometer of Cathcart, Wishart and McCall (16). The subject was instructed to work at the rate that seemed to him the most comfortable, against a resistance of 1 kgm. The work done was usually of the order of about 11,000 kgm. per hour. The revolutions of the ergometer wheel (and thus the rate of working) were recorded continuously during the experiment. By means of an electrical device, the two "Veeders" used for this purpose recorded alternately over 10 second intervals: records of any variation that might occur in the working rate could in this way be obtained. Each experiment in this series consisted of three parts, viz. (*a*) mental work alone, (*b*) muscular work alone, and (*c*) combined muscular and mental work, and each tripartite experiment was repeated at least three times. Each part of an experiment lasted on the first occasion for 15 minutes: on the two subsequent occasions the duration of each part was 5 minutes. The experiments were performed as a rule on separate days, with about a week's

interval between each experiment. The subjects in this group were instructed to perform the mental work as rapidly as possible.

The individual results of the first set of experiments of this series are given in Table I. The averages of the second and third sets, which were similar to those in Table I, are given in Table II and with these for comparison the averages of the first set.

TABLE I.

Subject	Muscular work alone				Mental work alone				Muscular and mental w[ork] combined			
	Max. B.P.	Percentage increase in B.P.	Max. P.R.	Percentage increase in P.R.	Max. B.P.	Percentage increase in B.P.	Max. P.R.	Percentage increase in P.R.	Max. B.P.	Percentage increase in B.P.	Max. P.R.	Pe[rcentage] inc[rease] in [P.R.]
A.	154	45·3	144	50	122	22	126	23·5	148	23·3	156	[illegible]
S.	168	50	160	196·3*	140	22·8	80	29	159	42	128	[illegible]
McK.	144	26·3	100	56·2	146	10·6	80	33·3	—	—	—	
C.	140	20·7	112	33·3	136	17·2	104	8·3	138	30·2	108	[illegible]
B.	144	33·3	120	43	126	31·2	90	37·4	144	30·9	102	[illegible]
M.	157	8·2	116	65·7	164	32·2	114	18·8	150	25	136	[illegible]
S. R.	130	25·5	112	47·3	136	9·7	102	21·4	154	22·2	140	[illegible]
M.	156	36·8	120	43	124	8·7	88	22·2	176	46·6	150	[illegible]
R.	148	15·6	106	20·5	136	65	100	8·7	148	23·3	108	[illegible]
P.	138	30·3	114	31	144	37·5	104	28·6	158	41·1	136	[illegible]
Av. percentage increases		29·2		58·6*		25·8		22·8		33·9		[illegible]

* If the exceptional reading of S. be omitted, average percentage rise is then 43 %.

TABLE II. Average percentage increases in pulse-rate and blood-pressure.

	Pulse-rate			Blood-pressure		
	Muscular work alone	Mental work alone	Mental and muscular combined	Muscular work alone	Mental work alone	Mental and muscular combined
1st set	43	23	62	29	26	34
2nd ,,	43	22	39	26	22	27
3rd ,,	41	19	46	24	14	31

It will be observed that the values for the percentage increases in blood-pressure diminish in the two latter sets of experiments, and this fall is rather greater in the case of mental work alone.

The pulse-rate also rose during mental work alone, in all the experiments of this group, but it showed a less close correspondence with the rise in pulse-rate occurring during moderate muscular work than did the corresponding changes in blood-pressure. It will be observed that the rise in pulse-rate is not so marked in the later experiments.

Where mental and muscle work are combined, the rise in pulse-rate and blood-pressure tends, in a majority of cases, to be higher than when either mental or muscular work is performed alone (*vide* Tables I and II).

The diminished extent of the rise in pulse-rate and blood-pressure in the later experiments suggested that the increases recorded might be

due partly or even wholly to emotional factors. The second series of experiments was therefore initiated in a group of five laboratory workers, who might be expected to experience, and as a matter of fact did experience, little emotional disturbance of any kind in tests of this sort

Series II. In the second series of experiments with five laboratory workers, mental work only was undertaken. The duration of the experiments was from 5 to 15 minutes The position of the subject was varied, in some cases the subject sat on a stool, in others at ease in an armchair, and in others, he lay recumbent on a bed The object of the last two positions was to secure as complete muscular relaxation as possible The instructions to the subject varied: in some instances, it was to add as rapidly as possible, in others to add at a comfortable rate Since the blood-pressure and pulse-rate might conceivably be influenced apart from definite mental effort, by *e g.* articulatory movements ("subvocal" during additions, vocal while calling out the results) or by the ocular movements of fixation of the figures on the moving paper, control experiments were performed in which the subject was instructed simply to read aloud certain of the figures as they appeared, or merely to call out numbers while keeping his eyes closed.

Twenty-one experiments, consisting, as a rule, in adding for 5 minutes in the manner already described, were done by them, together with ten controls. In all 21 experiments a rise occurred in blood-pressure, and in 19 out of 21 a rise occurred also in pulse rate (Table III A) Of the two experiments in which the pulse-rate failed to rise, in one the pulse had certainly not fallen to its resting level before the start, and in the other this was also probably the case In only two of the experiments was there a subjective record of anything like excitement In the control experiments (Table III B), which were performed with the object already stated, viz. to eliminate the effect of muscular tension, and of movements of articulation and ocular fixation, a rise in blood pressure occurred in six out of ten experiments, but it was much less on the average than the changes produced by mental work (see Table III C) Similarly with the pulse-rate, which showed a rise in only four cases out of ten, and then of much less magnitude than in the mental work experiments It appears, then, that adding performed while in a state of as complete muscular relaxation as it was possible to obtain, *i.e.* in the recumbent position in a bed, did not fail to produce a rise in pulse-rate and blood-pressure. On the other hand, when no addition was done, the subject simply reading aloud figures from the moving paper, little or no rise occurred.

Series III. A third group of subjects, consisting of eight women

TABLE V. Blood-pressure and pulse-rate during mental work.
First series of experiments.

Subject	Max. rise of B.P.	Time to reach max. in mins.	B.P. at end of 14 mins.	Max. rise of P.R.	Time to reach max. in mins.	P.R. at end of 15 mins.
A.	22	5	112	22	8½	120
S.	26	1½	136	18	1	72
D. M.	30	1¼	112	22	1¾	72
M.	40	1	152	18	2	112
McK.	14	3	138	20	6	70
D. M.	8	1	110	16	1½	86
J. R.	12	½	126	18	3	92
P.	40	2½	128	20	3	96
R.	8	2	132	8	1½	96
C.	16	1½	118	8	1	96

attaining a maximum in from 1 to 3 minutes, and fell towards the end of the work-period.

CONCLUSIONS.

(1) Mental work produces an increase in pulse-rate and blood-pressure.

(2) The increase is independent of emotional factors.

(3) The increase is not accounted for by movements of the articulatory muscles, or by known muscle-tensions.

(4) In combined mental and muscular work, the increases in pulse-rate and blood-pressure are greater as a rule than in mental or muscular work performed singly.

(5) In the case of the women students, the pulse-rate increased proportionately twice as much as the blood-pressure: whereas in the male students, the proportionate increases in blood-pressure and pulse-rate were fairly similar.

I wish to acknowledge my indebtedness to Prof. Cathcart, under whose supervision the work was done, and to the laboratory workers and students who so readily acted as subjects.

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HYPERGLYCÆMIC AND PHLORHIZIN GLYCOSURIA IN THE HEART-LUNG-KIDNEY PREPARATION. By S. de BOER (*Fellow of the Rockefeller Foundation*) and E. B. VERNEY (*Beit Memorial Research Fellow*).

(*From the Institute of Physiology, University College, London.*)

The heart-lung-kidney preparation seemed to us an eminently suitable one in which to study the excretion of glucose under conditions of (*a*) hyperglycæmia and (*b*) phlorhizinæmia. Thus the experiments fall naturally into two groups.

In the first series we have investigated the threshold level of the blood sugar and studied the effects of variations in blood-pressure on this level, as well as the variations which ensue in the percentage and absolute amounts of sugar excreted during pressure diuresis when the blood sugar content is above this threshold. In man this threshold lies between 0·17 p.c. and 0·30 p.c., the latter being an extreme figure noted by Graham(2). Rose(3) gives 0·25 p.c. as the leak point in rabbits.

The secretion of sugar in animals during profuse diuresis was first observed by Bock and Hoffmann(4), who infused large quantities of 1 p.c. sodium chloride into the peripheral end of the carotid artery of the rabbit. Since no blood sugar estimations were made in their experiments, it is impossible to say whether the glycosuria they observed was not due to a hyperglycæmia resulting from stimulation of the medulla by the hypertonic salt, rather than to an absolute lowering of the threshold by the extreme diuresis. This possibility in fact they recognise. Underhill and Closson(5) later showed that the intravenous infusion of hypertonic salt was accompanied by a polyuria and glycosuria which they attributed to an increased permeability of the kidney to sugar under these conditions. The blood sugar was below normal. Again Rose(3) showed that diuretin in rabbits was capable of causing a glycosuria even if the blood sugar was 0·09 p.c. below the normal leak point (0·25 p.c.). There is, then, already evidence that diuresis brought about by various means in the intact animal lowers the threshold of the kidney for sugar. We have been able to strengthen this evidence by experiments performed on the isolated organ.

When the blood sugar rises above the leak point value, sugar, as is well known, is passed in the urine in a concentrated form, the percentage varying inversely, and the absolute amount directly as the rate of urine flow (6). This is illustrated in the figures given by Pollak (7) in Exp. XI (adrenalin glycosuria in the rabbit), but the relation is not quite definite probably on account of the comparatively small changes in velocity of urine flow, and of the small volumes of consecutive samples of urine in relation to which the dead space of the kidneys and ureters gives rise to a correspondingly large source of error. The same general, though none too close, relation between the rate of urinary flow and the percentage and absolute amounts of sugar in the urine is shown in the experiments of Loewi (8), who investigated the effect of superimposing a diuresis elicited by the subcutaneous injection of 100 c.c. 10 p.c. $NaNO_3$ in dogs on a glycosuria already existing and brought about either by removal of the pancreas or by the intravenous injection of strong solutions of glucose. In our experiments the rate of urinary flow can be directly controlled and varied immediately at will by raising or lowering the blood-pressure (9). The changes in the composition of the urine tend therefore to be sharply defined.

The second series of experiments recorded in this paper deals with the action of phlorhizin on the isolated kidney. The evidence in favour of the "Elimination theory"[1] is already overwhelming (10), but it seemed to us necessary to investigate the effects of this body on the isolated kidney for two reasons, firstly because Pavy, Brodie and Siau (11), perfusing the organ by means of a pump, came to a conclusion contrary to that generally accepted, and secondly, because the effect on the absolute amount of sugar secreted of a diuresis superimposed on a phlorhizin diuresis is still *sub judice*.

Pavy, Brodie and Siau concluded from their perfusion experiments that the amount of sugar appearing in the urine was too great to be accounted for by a simple lowering of the threshold of the kidney to sugar, and suggested that it was manufactured by the organ from some preformed constituent brought to it by the blood. The large quantity of phlorhizin they employed, however, was amply sufficient to account for the excess of sugar collected. Furthermore, their experiments were complicated by the addition of large quantities of chloral to the blood.

Loewi (8) states that in the phlorhizinised dog a diuresis produced by the intravenous injection of hypertonic sodium nitrate or sodium sulphate

[1] For a short and excellent critical review of the four possible theories of the action of phlorhizin, see the paper by Erlandsen (10).

produces no increase in the absolute amount of sugar secreted. It is to be noted, however, that blood sugar determinations were not made. In a further paper (12) he came to the same conclusion although in Exp. VII there is as a matter of fact a well-marked rise in the total sugar eliminated at the height of diuresis—viz. from 1·03 to 1·42 gms. per hour. The percentage of sugar in the urine varied inversely as the urinary flow. Knopf (13) comes to the same conclusion as Loewi with regard to the amount of sugar eliminated in diuresis in the phlorhizinised dog due to urea, whilst Weber (14) states that salts and purines cause no increase under these conditions. The effect of changes in blood-pressure on the percentage and total sugar secreted has, as far as we are aware, not been investigated.

Method. Dogs were used for the experiments and the heart-lung-kidney prepared according to a method described elsewhere (1). The lungs were ventilated with warm air saturated with water vapour and the experiments were consequently performed under conditions of extreme acapnia. The blood sugar estimations were made according to the method devised by Maclean (15), 1 c.c. of blood being taken for each determination. The urine was analysed quantitatively for glucose by Benedict's method (10).

First series of experiments. Three concordant experiments were made. The results of one are given in Fig. 1, and those of another in Table 1.

TABLE I.

Time	Blood sugar %	T. °C.	B.P. mm. Hg.	Blood flow c.c. per min.	Urine flow c.c./15 mins.	Urine sugar %	Urine sugar mgms./15 mins.
2.25	0·04	35·3	100	72	9·7	Nil	Nil
2·26. Add 3 gms glucose in 30 c.c. 0·9 % NaCl.							
2·35	0·26	35·3	95	89	10·9	Nil	Nil
3·12	0·23	35·2	120	94	14·5	Trace	—
3·32	0·20	35·2	120	94	11·8	Trace	—
3·42 Add 3 gms. glucose in 30 c.c. 0·9 % NaCl.							
3·55	0·37	35	128	94	18·7	1·04	194
4·10	0·36	35	128	91	10·5	1·02	168
5·00	0·33	35	90	70	0·7	1·52	11

In interpreting the results of these experiments we must remember that the concentration of sugar in the blood is falling continuously, and that this fact tends to annul any increase in concentration in the urine which might occur in a urine sample procured at a low blood-pressure compared to the immediately preceding one procured at a high. Conversely the fall in concentration in the urine following a period of oliguria is accentuated. Bearing these considerations in mind, we think these

experimeents show fairly conclusively that variations in the rate of urinary flow determined by changes in blood-pressure are accompanied

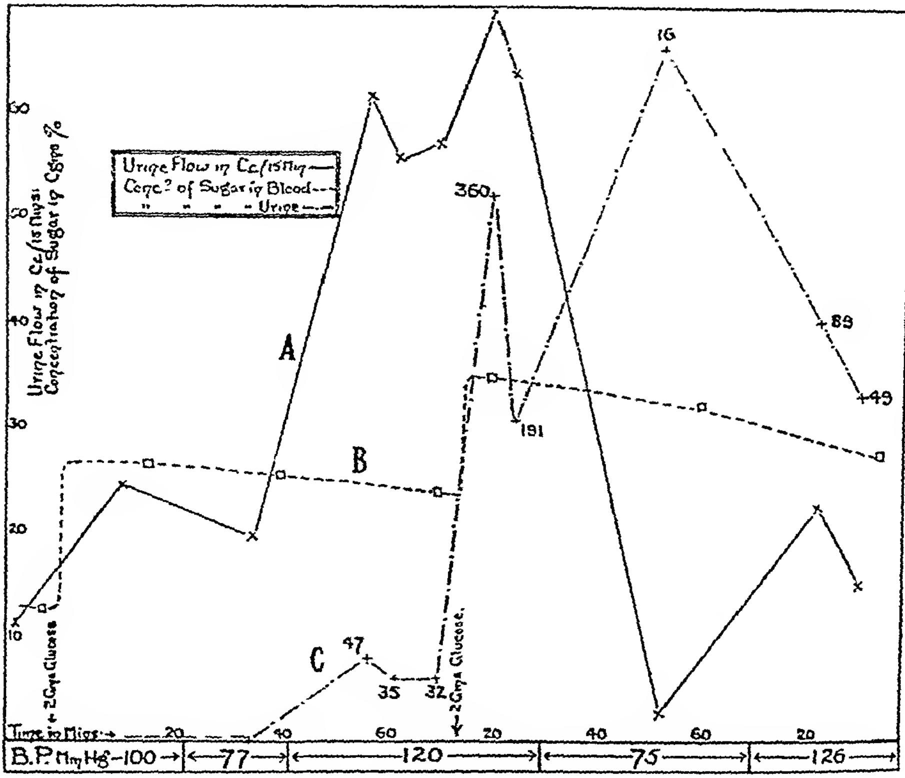

Fig. 1. *A.* Urine flow in c.c. per 15 mins. *B.* Blood sugar %. *C.* Urine sugar %. The figures on the urine sugar curve represent the absolute amounts of sugar (in mgms.) secreted in the 15 mins. At the point indicated by the arrow 2 gms. glucose were added to the blood.

by a change in the same sense in the absolute amount of sugar secreted. The change in concentration of sugar in the urine is always towards that of the blood when the rate of urine is increased and *vice versa.* When the blood sugar is below the threshold value a pressure diuresis causes the appearance of sugar in the urine, and when above the threshold value a fall in the already existing concentration. The threshold for sugar is seen to be about 0·25 p.c. (See Fig. 1.)

Second series of experiments. Here we have investigated firstly the relation between the amount of sugar disappearing from the blood and that appearing in the urine in a given interval of time after the addition of phlorhizin, and secondly the effect of changes in blood-pressure on the

percentage and absolute amounts of sugar eliminated under these conditions.

For our first purpose we must know (*a*) the volume of blood circulating over the given period of time and (*b*) the fall in the percentage of blood sugar over and above that due to glycolysis and to the metabolic requirements of the heart, lungs, kidney and blood. The volume of blood at any given time cannot be determined exactly but can be computed from estimations of that remaining in the heart, lungs and cannulæ at the end of the experiment. These were washed through with normal saline and the volume of blood present calculated by comparing the hæmoglobin content of the diluted sample with that of a specimen of whole blood taken at the end of the experiment. On one occasion we arrived at the figure 60 c.c., on another 70 c.c. The volume of the remainder of the apparatus was determined, and so a constant was arrived at which, added to the amount of blood in the venous reservoir (graduated) gave the total volume of blood circulating at any given time. Figures so obtained are not accurate but it seems unlikely that a greater error than ± 50 c.c. is involved.

The fall in blood volume is very gradual till towards the end of an experiment, when owing to the onset of pulmonary œdema the fall becomes very rapid. Our observations have all been made before the onset of this condition.

The next difficulty arises when we consider that there is normally a marked and continuous fall in the blood sugar in this preparation. This fall must be due to the sum of various factors, namely, glycolysis and the sugar consumption of the heart, lungs, kidney and blood. We therefore made control blood sugar estimations in this preparation and plotted the percentages of sugar found against time. The variations in the rate of fall were not nearly as great as we had anticipated and it was possible

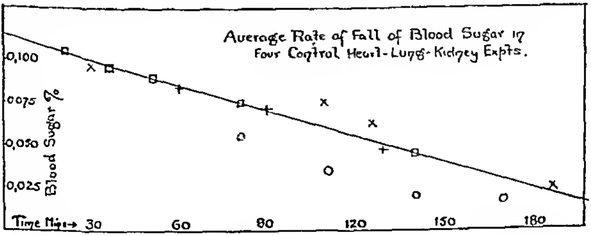

Fig. 2.

to construct a curve which defined fairly accurately the rate of fall in these control curves (Fig. 2). We can only conclude from this that differences in the sugar consumption of the tissues in this preparation under varying degrees of work and in the acapnic state are small compared to the amount of sugar which disappears as the result of some fermentative change.

The addition of phlorhizin caused a very well-marked change in the slope of this normal curve, as is shown in Figs. 3 and 4. The shaded

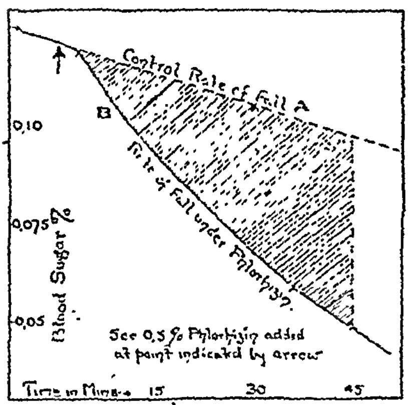

Fig. 3.

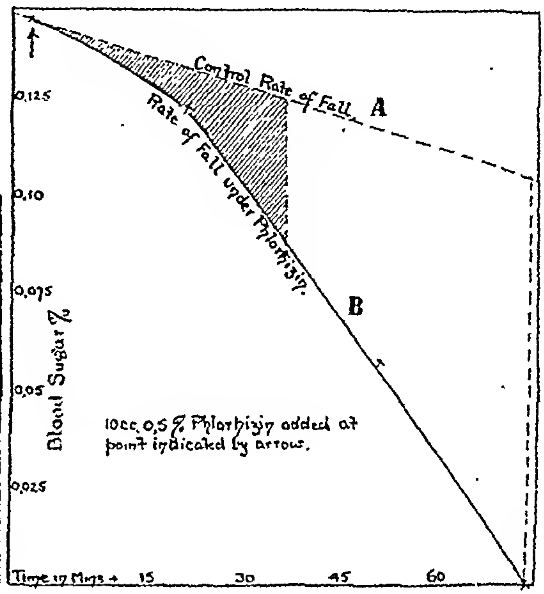

Fig. 4.

Figs. 3 and 4. Rate of fall of blood sugar. *A*. Control rate. *B*=rate under phlorhizin. Phlorhizin added at the arrow, 5 c.c. in Fig. 3, 10 c.c in Fig. 4. The shaded area represents the "extra" sugar disappearing from the blood.

area between the two curves is to be taken as representing the extra sugar which disappeared from the blood over this period of time as the result of the addition of phlorhizin. In Fig. 3 the extra sugar disappearing in the 45-minute period is 350 mgms., and the amount of sugar actually collected in the urine over this period of time 328 mgms. In the experiment from which Fig. 4 is taken these amounts were 350 and 375 mgms. respectively, over a period of 39 minutes. At the end of this period 250 c.c. normal saline were added and the percentage of sugar in the urine fell to a lower figure than could be estimated. It was thus impossible to take a further period and make a corresponding comparison

between the extra sugar disappearing from the blood and that appearing in the urine. We think, however, that the experiments do show that phlorhizin causes an appearance of sugar in the urine in amounts which bear a rough quantitative relation to the decrement in blood sugar over and above that disappearing normally over the same interval of time. The addition of phlorhizin caused no increase in the blood flow through the kidney, a fact which confirms the experience of other investigators (11, 17, 18).

The next diagram (Fig. 5) is of an experiment in which the percentage and absolute amounts of sugar secreted are compared at different rates of urinary secretion brought about by variations in blood-pressure.

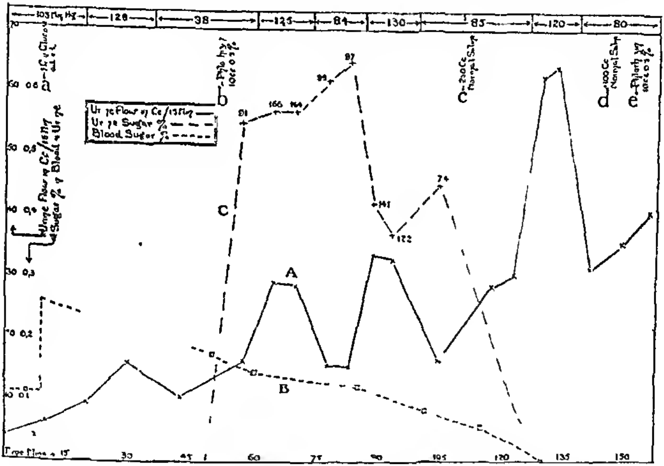

Fig 5 The effect of variations in blood pressure on the excretion of sugar after phlorhizin. A = urine flow in c c. per 15 minutes B = blood sugar %. C = urine sugar %. The figures on the urine sugar curve represent the absolute amounts of sugar (in mgms.) secreted in the 15 mins a 1 gm. glucose added, b 10 c c 0 5 % phlorhizin; c 250 c c. normal saline; d, 100 c c. normal saline; e, 10 c c 0 5 % phlorhizin

The results are plotted against time. The figures on the urine sugar curve represent the absolute amounts of sugar secreted in mgms. per 15 minutes. It will be seen that an increased rate of urinary flow is accompanied by a fall in concentration of sugar in the urine and by an increase in the absolute amount and *vice versa*. It will be seen further that the

experiment continued successfully for a period of half an hour after the blood sugar had fallen to zero. The urinary sugar percentage fell to a mere trace about the same time and although this trace did not disappear a further addition of phlorhizin completely failed to cause any increase in it, the blood sugar being already at zero. A further experiment gave results similar to those obtained in Fig. 5 and so its record is omitted.

CONCLUSIONS.

1. The isolated mammalian kidney shows a threshold level for glucose. This level in the dog is about 0·25 p.c.

2. The threshold can be lowered by an increase in the rate of urinary flow elicited by a rise in blood-pressure.

3. When the blood contains more than the threshold amount of sugar, changes in the rate of urinary flow brought about by changes in blood-pressure, are accompanied by inverse variations in the percentage amount and direct variations in the absolute amount of sugar secreted.

4. The amount of sugar appearing in the urine after the addition of phlorhizin to the circulating blood can be completely accounted for by the fall in blood sugar over the same period of time.

5. Changes in blood-pressure affect the percentage and absolute output of sugar in the heart-lung-kidney after the addition of phlorhizin in exactly the same way as when the percentage of sugar in the blood is artificially raised above the threshold.

We are much indebted to Prof. Starling for his help and advice throughout this investigation.

The expenses of this research were defrayed by grants to us from the Rockefeller Foundation (to S. de B.) and from the Government Grant Committee of the Royal Society (to E. B. V.).

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THE HEAT PRODUCTION OF MUSCLES TREATED WITH CAFFEIN OR SUBJECTED TO PROLONGED DISCONTINUOUS STIMULATION. BY W HARTREE[1] AND A V HILL

(*From the Physiological Laboratories, Cambridge, and University College, London*)

THE direct action of caffein—which, in some ways, is not unlike that of veratrin—on cross striated muscles has long been known An account of the previous work on the subject is given in Heffter's *Handbuch* (1) Under the influence of caffein in low concentration there is said to be an increase in the isotonic contraction, but the muscles are more easily fatigued, at higher concentrations, however, *e g* of 1 2000 or more, destructive changes are seen under the microscope to take place inside the fibres of a frog's muscle, and the muscle itself gradually shortens and finally becomes rigid and inexcitable, all the phenomena being similar to those occurring in the case of rigor produced by heat, distilled water or chloroform It is natural to associate the contracture and rigor with the gradual formation of lactic acid, and Ransom (2) in 1911 found that lactic acid is indeed liberated in large quantities in muscle suspended in Ringer's solution containing caffein Meyerhof (3) recently investigated more fully the action of caffein in increasing (*a*) the oxygen consumption and (*b*) the lactic acid formation in cut up frog's muscle when in oxygen, the oxygen consumption is increased up to 100 p c and Meyerhof states that other contracture producing substances do not raise the oxygen consumption In resting intact muscles caffein produces large amounts of lactic acid and at the same time raises the extent of the oxidation, the effect, however, of caffein on oxidation is not consequent simply on the increase in the lactic acid formation, since a greater formation of lactic acid, produced by stimulation, raises the oxygen consumption less

Riesser and Neuschloss (4), working on the amount of lactacidogen present in muscles treated with caffein, found that the muscular contracture which occurs spontaneously after stronger doses, but only in conjunction with electrical stimulation after weaker ones, is associated

[1] Working for the Medical Research Council

experiment continued successfully for a period of half an hour after the blood sugar had fallen to zero. The urinary sugar percentage fell to a mere trace about the same time and although this trace did not disappear a further addition of phlorhizin completely failed to cause any increase in it, the blood sugar being already at zero. A further experiment gave results similar to those obtained in Fig. 5 and so its record is omitted.

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this latter heat as a false base, from which the recovery heat deflection (if any) after a stimulus can be measured; for it is found that in two or three minutes after a stimulus the actual deflection will be below the false base. This, of course, does not necessarily mean that the muscle is absorbing heat; it is much more probable that the spontaneous heat-production due to the caffein has, at least temporarily, been *diminished* as one result of the stimulus.

General description of experiments and results. The only muscle used was the sartorius of *Rana temp.* mounted on a thermopile in the usual way, with a thread from the ends attached to a tension lever (approximately isometric). The temperature was always between 15° and 16° C. A "stimulus" always means a tetanus of $\frac{1}{4}$ sec. The sensitivity of the galvanometer was so arranged that, with a normal muscle, a stimulus produced a maximum deflection of about 300 mm. (on a scale about $2\frac{1}{2}$ metres from the galvanometer); provision was made to observe up to 600 mm., since the deflection due to the prolonged heat production after giving caffein is often quite large, and it was sometimes required to give a stimulus when the muscle was already showing this effect of caffein. For this sensitivity it was usual to have from 100 to 150 ohms in series with the galvanometer (galvanometer and thermopile together from 20 to 30 ohms), so that the zero settled down comparatively quickly.

After the maximal stimulus had been determined, and one or two preliminary normal curves of galvanometer deflection had been taken, the caffein solution (in Ringer[1]) was given for about five minutes, as shown in detail below. Care was taken to put the caffein solution into the chamber at a temperature which was as nearly as possible equal to that of the water in the vacuum flask surrounding the chamber, in which case reliable readings could usually be taken in less than 20 minutes. In many cases the effect of the caffein was not observable till much later than this. If, however, the caffein be given too strong (more than about ·07 p.c.) the galvanometer spot will be observed going in the "heating" direction before the temperature in the chamber has sufficiently settled, in which case the first readings will be quite unreliable; if, on the other hand, the caffein be given too weak (less than about ·05 p.c.) it may be some hours before the effect of the caffein is observable.

In a few cases (*A* and *G* in the experiments quoted) there was no sign of spontaneous contraction (the galvanometer curves in these cases were remarkably smooth); the effect, however, of the caffein on the

[1] ·65 % NaCl, ·02 % KCl, ·025 % $CaCl_2$, ·015 % $NaHCO_3$, in distilled water; pH about 6·9.

heat-production was very obvious. In these cases, about three hours after the effect of the caffein has definitely started, as well as (in other cases) about half-an-hour after a spontaneous contraction has commenced, a further stimulus gives no observable tension and no appreciable rise in the rate of heat-production; although the latter may be quite considerable and (at least when the muscle is in oxygen) may continue for some hours. The tension also, after a spontaneous contraction, falls off only very slowly, and had never disappeared even after observations lasting six hours or more. It may be remarked that, in nearly every case (including others not shown here) the spontaneous contraction arises, if at all, 30 to 60 minutes after the rate of heat-production due to the caffein has reached its maximum.

At the end of a few of the experiments the muscle was thoroughly washed in Ringer's solution: this, however, never brought about any sign of revival. Further, when the muscle was subsequently heated with a comparatively strong current, as in taking a "control" curve, the resulting curve of galvanometer deflection was found to be identical with that taken in a similar manner after chloroform had been put into the chamber.

In these experiments the maximum heat-rate due to the effect of caffein (apart from that due to spontaneous contraction) lay, when the muscle was in nitrogen, between 2×10^{-4} and 4×10^{-4} cal. per gram per sec., when in oxygen between 10×10^{-4} and 12×10^{-4} cal. per gram per sec.

Effect of caffein on the initial heat and tension consequent on a stimulus. With the strength of caffein generally employed (about ·06 p.c.), which does not usually cause a spontaneous contraction, the first stimulus, after caffein had been given and removed from the chamber, always gave rise to a larger heat production than the normal. The actual results seem to depend on whether the muscle is in nitrogen[1] or in oxygen. In six different experiments in nitrogen the initial heat for the first stimulus after caffein was between 10 p.c. and 12 p.c. greater than before, whereas the maximum tension was in no case greater than before and in some cases was nearly 5 p.c. less. In four different experiments in oxygen the results were not so uniform as in nitrogen, but the effect was greater in every case. In (1) the tension was about 10 p.c. less and the heat about 19 p.c. more: in (2) the tension was the same and the heat about 22 p.c.

[1] The nitrogen used was not specially purified; it was taken from a cylinder and passed in small bubbles through a bottle of alkaline pyrogallol, but probably had at least 1 % of oxygen in it; recently boiled Ringer's solution was always used in the experiments in nitrogen.

more: in (3) and (4) the tension was a little more and the heat about 29 p.c. more.

In every case subsequent stimuli diminished both the tension and the heat, their ratio remaining nearly constant in some cases for a long time. It appears, therefore, that for the same maximum tension, the heat production after caffein, when the muscle is in nitrogen, is about 15 p.c. greater than before caffein, while, when the muscle is in oxygen, it is about 25 p.c. greater. This fact is clearly to be correlated with the marked diminution in the speed of relaxation of muscles stimulated after caffein. The prolonged relaxation is presumably associated with a prolonged initial breakdown process, analogous to, but smaller than, that occurring after veratrin. Some of the energy is spent in maintaining the contraction, not all of it in developing the contraction: consequently the ratio of heat to maximum tension is increased.

The prolonged heat-production. The prolonged production of heat occurring either spontaneously or after a stimulus, is shown in Figs. 1 and 2. These diagrams are not strictly the true curves of heat production, but the observed curves of galvanometer deflection. It would be possible, but extremely laborious, to analyse them as explained elsewhere (5) by means of a "control" curve, obtained by heating the muscle when dead, and thus to find the true curves of heat production; this, however, is unnecessary, since the curves shown give a very fair description of the actual heat production, and this description is the more correct the more nearly horizontal the curves are. When, however, the curves are rising or falling very fast, it must be remembered that the galvanometer deflection "lags" behind the heat production. In these experiments, the thermopiles and muscles were such that the "control" curve would fall from its maximum practically to zero in four to five minutes, a very large part of that fall occurring in the first two minutes; thus the observed galvanometer deflection, at any instant during an experiment, is due to all the heat which has been produced in the preceding five minutes, but nearly entirely to that part of it which has been produced in the preceding one or two minutes. It is only when the heat rate is altering very rapidly that the galvanometer curves shown here do not represent it almost exactly. The effect of a stimulus is to make a tall sharp peak in the curve, falling to about 5 p.c. of its height in five minutes, the corresponding recovery heat slowing down the return as compared with the "control" curve. These peaks have not been shown in the diagrams.

Although the ordinate of the curve at any instant does not, strictly speaking, represent the heat rate at that instant, the whole area of the

after the caffein was given; the heat rate had then not fallen to half its maximum. Heat scale: 1 mm. × 1 min. = $1{\cdot}4 \times 10^{-4}$ cal. per gram. Total heat can hardly be estimated in this case. No sign of spontaneous or permanent contraction.

Several conclusions may be drawn from these experiments:

(1) The spontaneous contraction, whether in oxygen or in nitrogen, appears always to be followed by, if not to cause, a rise in the rate of heat production.

(2) As Gasser and Hartree have shown (6), a stimulus, if it fails to produce heat, fails also to produce tension, and *vice versa*.

(3) The spontaneous heat production continues to occur (*s*, curves *D* and *F*), to a small degree, in nitrogen, after the muscle has become inexcitable: this is presumably to be attributed to continuing lactic acid production.

(4) The spontaneous heat production may continue to occur (*s*, curve *A*) to a considerable degree, in oxygen, after the muscle has become inexcitable: this is presumably to be attributed largely to continued oxidation, possible after the muscle has become inexcitable.

(5) The total spontaneous heat production in nitrogen is about $3\frac{1}{2}$ cal. per gram of muscle. Accepting Meyerhof's (7) value of 370 cal. per gram of lactic acid, this implies a formation of about ·9 p.c. of lactic acid in the muscles. This value, possible according to Meyerhof (3, p. 144, etc.) for cut up muscles in an alkaline phosphate medium, is high for an intact muscle in gas. The sartorius, however, as used, is very pure muscle substance, and might be expected to give higher values than the mixed muscles chopped off a frog's leg. It is possible, moreover, that traces of oxygen remaining in the nitrogen used may have allowed a small degree of oxidation. There would seem, in any case, to be no good reason for attributing the anaerobic spontaneous heat to anything other than the formation of lactic acid.

(6) The total spontaneous heat-production in oxygen is about 15 cal. per gram, several times greater than in nitrogen. There can be no question here that the main part of the heat is due to oxidative processes, as is shown by Meyerhof's (3) observation of a considerable oxygen consumption in muscles treated with caffein. Assuming an anaerobic heat production, the same as in section (5) above—due to the formation of lactic acid to the same maximum concentration—this leaves 11·5 cals. as due to oxidative processes. Employing Slater's (8) value (3874 cal.) as the heat of combustion of 1 gram of dissolved glycogen $(C_6H_{12}O_6)_n$, this implies the oxidation of about 0·3 p.c. of glycogen in the muscle. This is a perfectly possible value. It is striking

that the oxidative processes are so active in the muscle treated with caffein.

(7) The general type of the curves (Fig. 1) in oxygen is the same as that of the curves (Fig. 2) in nitrogen, in spite of the fact that four-fifths of the heat in the former case is due to a prócess (oxidation) which cannot occur in the latter. It is obvious therefore that the oxidation in question is excited by, and is dependent upon, the anaerobic changes: the oxidation is not a spontaneous affair, it is a necessary consequcnce of the spontaneous activity induced by caffein: it is in that sense a recovery process. Oxidation, as usual, is consequent on, but does not determine activity.

(8) The spontaneously developing contracture is presumably due to the increasing concentration of lactic acid.

The heat production during prolonged discontinuous stimulation of isolated muscle. The preceding experiments on the prolonged heat production due to the effect of caffein suggested that a similar method might be employed to find the total heat produced by a muscle subjected to stimuli at short intervals and continued as long as the muscle is capable of producing tension. The comparison of the results of such stimulation with those of caffein might throw further light on the action of the latter.

The stimuli were given at regular intervals, and records made of the maximum and minimum readings of the galvanometer. Seeing that the total heat is represented by the total area of the curve of galvanometer deflection, it is necessary to know how the spot moves in the interval between one minimum reading and the next. For this purpose, if the intervals be half minutes, as was the case in both the examples shown in Fig. 3, readings are taken every five seconds during several intervals, and a curve is formed as shown in the small diagram inserted in Fig. 3. In this case (and for shorter intervals), the curve is so nearly a triangle that its "mean height" (to give the same area on the same base) is very nearly half way between the minimum and the maximum; for longer intervals, however, the "mean height" will come much nearer the minimum than the maximum. The broken lines in Fig. 3 are drawn at these calculated "mean heights," and the areas of the corresponding curves give the total heat on a scale determined by giving the muscle a known amount of heat, when dead, and measuring the total area of the "control" curve caused by that heat.

After a few (six or eight) stimuli, the maximum and minimum readings become nearly steady and diminish gradually as time proceeds. The

maximum divergence from a smooth curve was not usually more than twice the thickness of the line in the diagram.

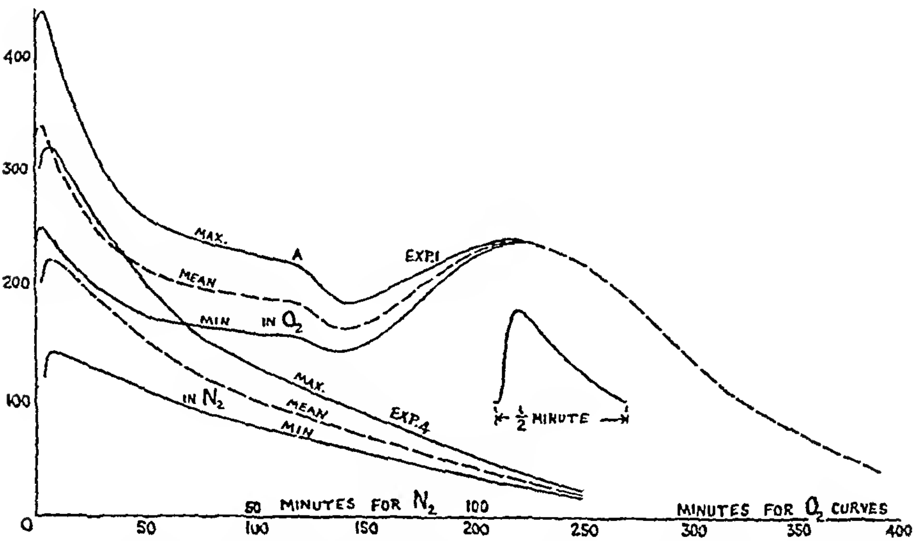

Fig. 3. Heat production (galvanometer deflection) in muscle excited discontinuously to complete fatigue by a long succession of short tetanic stimuli every half minute. Exp. 1, Table III, in oxygen; Exp. 4, Table III in nitrogen. The scale of time is twice as great in the nitrogen experiment. Each stimulus causes a deflection of the type shown in the small diagram inset; in each experiment the upper curve ("max.") is drawn through the tops of the successive deflections, the lower curve ("min.") through their bottoms, the middle curve ("mean") approximately half way between the other two.

These curves represent the sum of (*a*) the initial heat and (*b*) the delayed heat, arising from the stimuli, and these heats cannot be separated by this method; moreover, the rise due to a stimulus between minimum and maximum is not proportional to the initial heat, since this rise depends very much on the rate at which the spot is moving backwards at the time the stimulus is given, which is very different at different points along the curve. The results of five experiments are given below.

TABLE III.

(*a*) *Muscle in oxygen.*

Exp. 1. In O_2 at 14·5° C. Upper three curves, Fig. 3. Heat scale: 1 mm. × 1 min. $= 2{\cdot}2 \times 10^{-4}$ cal. per gram. Total heat about 15 cal. per gram. Tetanus $\frac{1}{4}$ sec., given every $\frac{1}{2}$ min. for 220 mins., by which time the tension and initial heat on stimulus had become very small, though the heat production was still quite large and continued for some time longer. At the point marked *A* the maximum tension due to a stimulus fell rather sharply owing to some unknown cause.

Exp. 2. In O_2 at 15·0° C. Tetanus ½ sec. given every min. for 3 hours, then every ½ min. for 2 hours; still considerable tension on stimulus at end. Total heat about 18 cal. per gram, including an allowance for the further heat after the end of the observations.

Exp. 3. In O_2 at 12·6° C. Tetanus ½ sec. given every ½ min. for about 3 hours. "Mean height" curve falls faster than before, and little tension on stimulus at end. Total heat about 8 cal. per gram.

(*b*) *Muscle in nitrogen.*

Exp. 4. In N_2 at 15·0° C. Tetanus ½ sec. given every ½ min. for about 2 hours: lower curves, Fig. 3. Heat scale: 1 mm. × 1 min. $= 2 \cdot 2 \times 10^{-4}$ cal. per gram. Total heat about 2½ cal. per gram.

Exp. 5. In N_2 at 13·5° C. Tetanus ½ sec. given every min. for about 3 hours. Considerable tension left at end and zero doubtful. Total heat roughly 3½ cal. per gram.

From these experiments we may conclude that:

(1) As was to be expected, the total heat production, to complete exhaustion, is very much greater in oxygen than in nitrogen: and the falling off of the heat production, due to oncoming fatigue, much less rapid in oxygen..

(2) The total heat in oxygen may be as high as 15 to 18 cal. per gram, *i.e.* of exactly the same order of size as the spontaneous heat in oxygen, after treatment with caffein. With too frequent stimulation (*e.g.* Exp. 3), leading to early exhaustion, or under unfavourable circumstances, it may be much less.

(3) The total heat in nitrogen is about 3 cal. per gram, *i.e.* of exactly the same order of size as the spontaneous heat in nitrogen after treatment with caffein.

(4) Therefore, that the action of caffein on muscle is merely to release the chemical processes normally induced by stimulation.

In an earlier paper A. V. Hill (9) made an indirect estimate of the total heat production of muscles kept in oxygen, air, or Ringer's solution: the principle adopted was to measure the total tension set up in a long series of twitches, due to stimuli every 2 or 10 secs., and then, knowing approximately the ratio of tension to heat production in a normal muscle, to calculate the heat. The method was admittedly rough, but should give the right order of quantities. In oxygen the results were variable, ranging from 5·6 to 47·0 cal. per gram, with a mean value of about 17 cal.: naturally not much reliance can be placed on this mean, but it is of the same order of size as we have found above. In air two values were 1·4 and 1·6 cal., somewhat less than we have found here in nitrogen. There is little recovery in muscles stimulated so frequently in air. In oxygenated Ringer's solution the results were very consistent,

having a mean value of 33 cal. Muscles unquestionably live longer and function more consistently in oxygenated Ringer's solution than in oxygen, especially when on a thermopile in oxygen, and there is no difficulty in supposing the muscle to possess enough oxidisable material (0·8 p.c. carbohydrate or lactic acid) to yield about 33 cal. per gram on oxidation. Under the conditions, however, of our present experiments it does not seem possible to attain such high values, at any rate with autumn frogs.

Technical Details.

Since the method employed in these experiments is novel, it is desirable to include a short critical discussion of its application. It cannot be used indiscriminately. Its accuracy evidently depends very largely on the steadiness of the galvanometer zero. In this discussion it is necessary to distinguish between the position of the spot on the scale when the thermopile is cut out, which may be called the "absolute" zero, and the position of the spot when the thermopile is in circuit with the galvanometer, and there has been no stimulus given for a considerable time, which may, for shortness, be called the "thermopile" zero. When the galvanometer has a magnetic control the "absolute" zero will, of course, be affected by any magnetic disturbances, but with sufficiently good shielding the effects of these can be reduced to very small amounts, apart from exceptional circumstances. In any case, the "absolute" zero can be frequently observed during a long series of readings, by simply breaking the galvanometer circuit: in this way the actual readings may be corrected for any displacement observed. In the above experiments all the readings were taken from the "absolute" zero, and there is no possibility of error arising from its motion.

The "thermopile" zero is much more troublesome to deal with; its motion may be practically impossible to detect, and this may give rise to errors. The first consideration is that of uniformity of temperature within the chamber. To secure this it is necessary not only to wait for at least 1½ hours after the muscle chamber has been put into the vacuum flask, but the temperature also of the water in the flask must be kept as nearly constant as possible. For this reason the experiments have been tried only at, or a little above, room temperature, constancy of temperature being obtained approximately by the use of heating coils in the flask.

A more subtle error of this kind may arise as follows. In practically every case in which a muscle is in good condition and has been at rest for a long time, the "thermopile" zero is displaced from the "absolute" zero in the "cooling" direction by an amount which is often by no means negligible. Further, it seems to be practically an invariable rule that if the "thermopile" zero be moving in the "heating" direction at a time which is sufficiently long after a stimulus for the recovery heat effect to be negligible, then the muscle is either dying or, at least, in very uncongenial surroundings. Even if the "thermopile" zero be fairly steady after setting up the muscle and waiting sufficiently long, and one or two stimuli be given, then in half an hour or so, after the effect of the recovery heat has become negligible, this zero will usually be found displaced in the "cooling" direction. No satisfactory explanation of these displacements of zero is yet available: the matter is being further investigated.

Some agency is clearly keeping the muscle slightly cooler than its surroundings. If this be the case it is obvious that, in the course of a long-continued heat production, an error may arise which cannot easily be detected, although such an error would be obvious at the end of a record after a *single* stimulus (such as is used to determine the *recovery heat*) since the "thermopile" zero is then under close observation.

In all the experiments referred to above, the total heat production, although spread out over a long time, takes place at a comparatively high rate, hence a large resistance must be used in series with the galvanometer, which diminishes the effect of these errors. The end part, however, of each record is always more doubtful especially as it is impossible to continue the readings until the heat production is entirely over. If the latter could be done, an estimate could be made at least of the final error of the "thermopile" zero, and some allowance could be made *for it*. As *it is, the best that can be done (and it is a very* useful check) is to make the fairly reasonable assumption that, after a sufficiently long time, the real heat rate is asymptotically approaching the value zero: this is justified by the fact that no case has been observed in which the spot, towards the end of an experiment, did not move at a diminishing rate, and it can hardly be supposed that a final *steady* state could be reached in which the real heat rate is anything very different from zero. Thus if, as sometimes happened, the final readings (from "absolute" zero) were negative and getting more negative but at a decreasing rate, the curve tending towards a fairly obvious asymptote, then this assumption was taken to provide a truer base for the real heat rate than the horizontal line of "absolute" zero reading, the difference being presumably due to "thermopile" zero error. This error, if appreciable at the start of the experiment, can easily be observed by noting the reading at least half an hour after a stimulus (if the temperature be not less than 15° C.: at lower temperatures the recovery heat will still be apparent at that time), this gives the start, and the above asymptote gives the end, of a base line from which the real heat rate should be read, when corrected as far as possible for "thermopile" zero error.

If the use of such corrections be to add 10 % or more to the heat (uncorrected) up to any point, the experiment must be classed as doubtful, since this limit was exceeded in only one[1] out of six experiments on repeated stimuli, and in none out of eight on the effect of caffein it appears to be possible, with sufficient care, to obtain fairly reliable results in experiments of this nature.

SUMMARY

1. In frog's muscle treated for a few minutes with caffein (about ·05 p.c.) there is a prolonged spontaneous liberation of heat, lasting till after the muscle has become completely inexcitable. In nitrogen this amounts altogether to about 3 cal. per gram of muscle, in oxygen to about 15 cal.

2. In frog's muscle stimulated with a short tetanus every half minute, till completely inexcitable, there is a total production of heat in nitrogen of about 3 cal. per gram, in oxygen of about 16 cal. The heat production is maintained much longer in oxygen than in nitrogen. The agreement of these values, obtained by a similar technique, with those resulting from treatment with caffein suggests that the action of caffein on muscle is merely to release, slowly and continuously, the chemical processes, anaerobic or oxidative, normally induced, suddenly and discontinuously, by stimulation.

3. The time course of the prolonged evolution of heat after caffein

[1] The results of this experiment have not been included above.

is the same, in general type, whether the muscle be in oxygen or in nitrogen, although it is of five times the magnitude in oxygen. This shows that, as is usual in muscle, the oxidative processes follow, and are consequent upon, the primary breakdown processes induced by activity.

4. In these experiments caffein appears, judging from the heat production, to liberate about 0·9 p.c. of lactic acid in the anaerobic muscle, and in oxygen, in addition, to cause the oxidation of about 0·3 p.c. of lactic acid or glycogen.

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THE LACTIC ACID IN THE BLOOD OF A RESTING MAN. By C. N. H. LONG[1], M.Sc.

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A STUDY of the work done on the lactic acid content of the blood of a resting man shows that there is some difference of opinion as to whether this acid is really present under true resting conditions. A number of observers employing several different methods have investigated the question. Berlinblau(1) estimated the acid as zinc salt and says it is a constant constituent of human blood. He gives the figure 8 mgrs. per 100 c.c. as the usual amount found. Salomon(2) using the same method states that lactic acid is absent during life but rapidly appears in the blood after death. Most of his determinations were made on hospital patients. Jerusalem(3) estimated the acid by oxidation with dilute permanganate solution, after the acid had been extracted with ether; the aldehyde formed was caught in bisulphite solution and estimated. Although he used large amounts of blood he could detect no lactic acid. This is remarkable as all other observers who have used an oxidative method have obtained positive results. Fries(4) using the same method as Jerusalem but chiefly on the blood of hospital patients found values varying from 9–63 mgrs. per 100 c.c. Ryffel(5) estimated the acid colorimetrically, by oxidation with strong sulphuric acid and the use of Schiff's reagent. His values were about 15 mgrs. per 100 c.c. Barcroft(6) using Ryffel's method found values in healthy young men of 12–19 mgrs. per 100 c.c. Clausen(7) greatly improved the technique of the permanganate method and states the usual amount in man is 15–32 mgrs. per 100 c.c. Barr, Himwich and Green(8) using Clausen's method found values of 14–25 mgrs. My own values using the same method show a value ranging from 10–20 mgrs. per 100 c.c.

At this point the criticism of Clauaen(7) on the estimation of lactic acid in blood by oxidative methods should be noted. He says: "It must be emphasised that biological fluids probably contain substances other than lactic acid which are extracted by ether and which yield bisulphite binding compounds on oxidation."

The bulk of the above evidence is in favour of lactic acid being a

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normal constituent of human blood, even under resting conditions, but in addition to Clausen's statements as to the possibility of other substances giving rise to aldehydes and also forming similar zinc compounds we have to consider the work of Lovatt Evans (9) on the formation of lactic acid in drawn blood by glycolysis. This glycolysis is easily prevented by the addition of small amounts of sodium fluoride, but none of the above workers except Barr, etc. (8), used this precaution and it is very likely that some at any rate of the lactic acid present may have had its origin from the blood sugar after the withdrawal of the blood sample.

Again some workers used the blood of hospital patients for their determinations. The lactic acid found under these conditions may have appeared as the result of some pathological changes in the subject and indeed the high values obtained by Fries (4) seem to suggest this.

Lastly, in all the foregoing investigations no special precautions were taken to see that the subject was in a true resting condition when the blood was withdrawn, since, in most cases, the results were comparative ones only. The movements of everyday life would probably account for the appearance of a certain amount in the blood. The possibility of this is emphasised by the work of Hartree and Hill (10) on the recovery heat production. They found that the recovery process in the isolated muscle appears to go on at a rate roughly proportional to the square of the concentration of the bodies produced during the preceding activity. Thus when the concentration of lactic acid produced by the stimulus is small the recovery process will be very slow. Now all muscular exertion liberates some lactic acid in the muscles, from which it tends to diffuse into the blood; its final or oxidative removal probably occurs mainly or entirely after its diffusion back again into the muscle. When the concentration of lactic acid in the muscles and blood is low the speed of the recovery process tending to remove it—being proportional to its square—will be extremely small, and the last phase of complete recovery will take a very long time. It is necessary therefore, in such experiments, that the subject should have been completely at rest for a long period before the sample of blood is taken.

Use of the thiophene test. Since the permanganate method estimates other substances as lactic acid besides the acid itself it was necessary to make use of a more specific test for lactic acid. The one used was that devised by Fletcher and Hopkins (11). These authors say that this test will detect 1 mgr. of lactic acid, but I have found that about $\frac{1}{2}$ mgr. of the acid can be detected in the form of its zinc or lithium salt.

The blood samples were taken from healthy young men who had had no strenuous exertion for some hours previously and who had lain down at least ½ hour before the blood was withdrawn The blood (venous) was fluorided and oxalated as soon as drawn 10 c c of the sample were taken and proteins removed by the method of Folin and Wu (12) Glucose was then removed from the filtrate by the method of Van Slyke (13) The solutions were then slowly evaporated to dryness on the water bath and the residue extracted with hot absolute alcohol The excess of alcohol was removed and the test performed on the residue

Blood, however, contains substances other than lactic acid which are extractable by alcohol and which might yield a positive thiophene test In order to see if this was occurring the test was performed on the following substances which are usually present in normal blood, viz urea, uric acid, cholestrol, amino acids, glucose, creatine aceto acetic acid, and acetone, etc None of these substances either in the solid state or in the alcohol gave a positive reaction It appears then that in the case of blood, at least, a positive thiophene test points to the presence of lactic acid itself

Experiments have been carried out in this manner on four different subjects and lactic acid appears to be a constant constituent of the blood even under resting conditions In several of the experiments an attempt was made roughly to estimate the amount of lactic acid indicated by the thiophene test and to compare the value obtained with that given by Clausen's method To do this the thiophene test was performed on known amounts of lactic acid (as zinc lactate) at the same time as it was carried out on the blood filtrate The colour obtained in the latter was then quickly matched against the standards and a rough estimate of the amount of lactic acid present was made

The figures found seem to indicate that one-half to three quarters of the values given by Clausen's method are due to the lactic acid itself, the remainder being due presumably to other hydroxy acids

TABLE I

Subject	Lactic acid content of blood Mgrs per 100 c c (Clausen)	Thiophene test (Qualitative)	(Quantitative) Mgrs per 100 c c
H L	17 5	Positive	12–15
A S P	35 6	Positive	—
S S	21 4	Positive	10–15
C N H L	17 5	Positive	10–15
C N H L	27 5	Positive	6 8

Effect of oxygen and carbon dioxide on the resting lactic acid If lactic acid be indeed a normal constituent of resting blood, as these thiophene

concentration of lactic acid in the muscles might be expected to occur. This steady concentration of lactic acid in the muscles would be accompanied by a corresponding lactic acid concentration in the blood, owing to the diffusion equilibrium existing between them. It would seem probable therefore that the "resting" lactic acid concentration found in the blood really is a genuine physiological effect, corresponding to that in the muscle, and determining the speed of the recovery processes of which the measure is the oxygen consumption.

Summary.

1. The thiophene test of Fletcher and Hopkins has been used to show the presence of lactic acid as a constant constituent of the blood of healthy young men at rest.

2. Attempts have been made to estimate roughly this amount by means of this test, and results show that $\frac{1}{2}$ to $\frac{3}{4}$ of the resting "lactic acid" estimated by Clausen's method is lactic acid itself, the rest being other substances which yield bisulphite binding compounds on oxidation.

3. The breathing of pure oxygen for an hour does not remove this resting lactic acid from the blood.

4. If 8–11 p.c. of carbon dioxide in pure oxygen is breathed for $\frac{1}{2}$ hour the blood no longer gives a positive thiophene test. The disappearance of the lactic acid is associated with a changed hydrogen ion concentration of the blood. This is in agreement with the results of Anrep and Cannan on the heart-lung preparation.

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EXPERIMENTAL HERMAPHRODITISM ON QUANTITATIVE LINES[1]. (Intratesticular ovarian transplantation by the method of Sand.) BY A. LIPSCHÜTZ, W. KRAUSE AND H. E. V. VOSS.

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STEINACH has shown in his feminisation and masculinisation experiments that the hormones of the ovary and of the testicle act differently; their hormonic effect is a *sex specific* one. Steinach's results have been confirmed by numerous authors such as Athias, Brandes, Goodale, Harms, Lipschütz, by Lillie and his co-workers Moore and Minoura, by Pézard, by Sand and by Zawadowsky(1). Steinach(2) made experiments also in which a simultaneous transplantation of ovary and testicle in mammals was performed; a hermaphroditic condition was thus obtained. Male guinea-pigs showed fully developed male characters and at the same time the mammary glands and the teats were highly developed as in a suckling female. Similar experiments with experimental hermaphroditism were made also by Sand(3) after the results of feminisation and masculinisation experiments had been published by Steinach. Great progress in the problem of experimental hermaphroditism has been made owing to the experimental work done by Sand by his new method of intratesticular ovarian transplantation(4).

The endocrine function of the sex gland has been studied during the last years also on quantitative lines. Pézard(5), in Gley's laboratory, has performed very exact experiments on the fowl. Lipschütz(6) and his co-workers have studied the quantitative aspect of the endocrine function of the sex gland in mammals; they showed that the fragments of testicle or of ovary which still suffice to condition a perfectly normal hormonic effect, may be very minute ones. Now the question arises, whether the hormonic effect and the condition of an ovarian or testicular fragment depends, if a state of experimental hermaphroditism is produced, upon the quantity of testicular or ovarian mass simultaneously present in the same animal. It is clear that this question is of great importance in relation to the problem of the antagonism between

[1] Partly communicated the 23 June and the 8 Dec. 1923 in the *Soc. de Biol. de Paris*. Specimens have been demonstrated at the Physiol. Congress, Edinburgh.

testicular and ovarian hormones, as discussed in the recent years by Steinach, Sand, Lipschütz, Moore and Lillie.

The intratesticular ovarian transplantation method of Sand provides a means of studying the question effectively. It is only necessary to vary the quantity of ovarian mass engrafted into the testicle and to examine whether an ovarian graft which is much below the normal ovarian quantity, will cause a similar hormonic effect to an ovarian fragment which is present by itself in the body of a female, and to examine the condition of the ovarian fragment. An experimental investigation on the lines indicated was undertaken. 36 male guinea-pigs operated on by Krause by the method of Sand at an age of 4–8 weeks, were observed during 1–7 months.

The quantities of ovary were varied in the following manner:

First group[1]: (1) an ovary into each testicle (I B); (2) an ovary into one testicle, the second testicle left intact (I C and II B); (3) the half of an ovary into the testicle, the second testicle left intact (I D and II C).

Second group[1]; (1) one testicle removed, two ovaries into the remaining testicle (I A); (2) one testicle removed, an ovary into the remaining testicle (II A).

We shall confine ourselves to a short general review, as detailed papers of Krause and of Voss will deal fully with the single experiments.

Twelve animals with a positive result have been observed.

TABLE.

First group: testicular mass not reduced.

	Quantity of ovary and testis	Weight of ♂ (gr.)	Weight of ♀ (gr.)	Duration of exp. in months	Beginning of hyper-trophy. Weeks after oper.	Maximal develop-ment of teats. Weeks after oper.	Total number of exps. 1 to 7 months
I B, 2	2 : 2	170	abt. 120	>6	7–8	17–18	5
I B, 6	2 : 2	155	200	>6	12–13	17–18	
I C, 1	1 : 2	320	140	7	18–19	20–21	11
II B, 1	1 : 2	290	adult	9 weeks	7–8	9	
I D, 3	½ : 2	240	240	6	13–14	15–16	14*
I D, 6	½ : 2	165	200	6	20–21	22–23	

* All the seven experiments with ovarian grafts from adults were negative.

Second group: testicular mass reduced.

I A, 1	2 : 1	255	200–250	>2	6–7	8–9	3
I A, 2	2 : 1	225		>2	6–7	8–9	
I A, 3	2 : 1	245		>2	6–7	8–9	
II A, 1	1 : 1	310	adult	<2	5–6	6–7	3
II A, 2	1 : 1	310	,,	>2	7	8	
II A, 3	1 : 1	295	,,	7	5–6	8	

[1] In the notes published in the *C. R. de la Soc. de Biol.* and in the *Deut. Med. Wochens.* (1923) the experiments were *grouped and marked in a different manner.*

In the first group a marked hypertrophy of the teats started about 2–4 months after the operation. The maximal length was attained about ½–3 months after beginning of growth. The length of the teats was up to 7 mm. corresponding to that of the teats of a suckling female; the length of teats in a normal male is about 1–2 mm. The shape of the teat in the engrafted males was also that characteristic of the normal female; the teat was very large at its basis. The area around the teats was protruded. Twice, a yellow doughy mass from the mammary gland was expressed; there was no secretion of milk as in many experiments of Steinach, Sand and Athias[1].

Some weeks after the maximal development was attained a decrease in size began; but the teats remained always much bigger than in normal males or virginal females.

Out of the six positive cases of the first group, two were implanted with only one half of an ovary. The degree of the feminine hormonic effect revealed itself independent of the engrafted quantity of the ovary. But possibly the time between the operation and the beginning of the hypertrophy depends upon the quantity engrafted; but nothing definite as yet can be said as to this.

As to the second group in which the mass of testicle has been reduced, all the cases were positive, without any exception. The feminine hormonic effect begun about six weeks after the operation and in about eight weeks the maximal development of the teats was attained, both when ovaries of young and adult females were engrafted. It seems clear from these experiments that reduction of testicular mass favours the hormonic effect of the engrafted ovary and diminishes the time of latency.

The penis and its accessory apparatus which in the guinea-pig are so sensitive to testicular disfunction, were always completely normal. The horny styles of the intromittent sac of the penis were as long as in normal animals, whereas even after postpuberal castration they undergo reduction, as it was known already to Steinach and as it has been studied especially by Lipschütz and Bormann. The mucosa of the intromittent sac had its normal epidermal excrescences, whereas in castrated animals the mucosa becomes smooth. The size of the penis was normal. To sum up: the external male sex characters of all the animals were as highly developed as in normal males notwithstanding the presence of highly developed female sex characters.

[1] In new experiments of Lipschütz and Voss, milk secretion was observed in many cases.

The dissection (Lipschütz) revealed the following. The operated testicle was mostly smaller than the normal one. But nevertheless spermatozoa were present in the epididymis. The operated testicle was mostly adherent to the abdominal wall in such a manner that a retention of the testicle was caused without spermatogenesis, this, as we see, being necessarily inhibited. The seminal vesicles and the prostate were highly developed as in normal adult males. The content of the seminal vesicles was examined as to coagulation. As was shown by Camus and Gley(7), the coagulation of the vesicular content is conditioned by prostatic secretion; coagulation can be caused when a very small quantity of prostatic fluid is added to the vesicular content. In the operated animals coagulation took place in less than one minute, though the fluid expressed from the prostatic gland was diluted by physiological solution. On the contrary, the content of the seminal vesicles in castrated animals, as was shown by Gley and Pézard(8) and as Lipschütz has observed in numerous cases, is a thin fluid and does not coagulate.

No examination of the psycho-sexual behaviour was made, since such observations are always very subjective and cannot serve as a basis for any quantitative statements.

The microscopical examination (Voss) revealed the ovarian graft in 11 out of the 12 positive cases. Follicles of different degrees of development were present. An ovarian graft was present, in accordance with a statement of Sand (4), also in some cases in which no feminine hormonic effect was to be seen, though the ovary was in a perfect condition[1].

Summary.

The statements of Steinach and of Sand that a simultaneous great development of male and female sex characters can be conditioned if testicle and ovary are present simultaneously in the same organism, was fully confirmed with guinea-pigs. Sand's method of intra-testicular ovarian transplantation was used. Out of 36 cases 12 were positive.

In those experiments in which both testicles were present, there was a positive result in 6 out of 30 operated animals. Those experiments in which one testicle was removed previously to ovarian transplantation, gave positive results in all the six operated cases.

The time of latency, *i.e.*, the time between the operation and the

[1] New experiments of Lipschütz and Voss, which will be published soon, gave an explanation of this phenomenon on the basis of the theory of the antagonism between male and female sex hormones.

beginning of hormonic effect, was considerably shorter in the experiments in which one of both testicles had been removed.

It seems highly probable, that the testicle inhibits by some kind of antagonism the survival and the hormonic effect of the ovarian graft. This antagonism does not exclude the survival and the hormonic effect of ovarian fragments, which are very small as compared with the normal ovarian mass or with the testicular mass simultaneously present.

There were some indications that in those experiments in which both testicles remained *in situ*, the time of latency of the feminine hormonic effect is shortened when more ovarian mass is engrafted. But no definite statements as to this could be made. The time of latency seems to be shorter, when an adult ovary is engrafted instead of an ovary of a young female.

All the seven experiments, in which the half of an ovary of adult animals has been engrafted into one of both testicles present, were negative, whereas out of seven similar experiments with half an ovary of young animals two gave positive results.

The statement of Sand was confirmed that the ovary engrafted into the testicle can survive without a feminine hormonic effect being produced. In one of these negative cases the engrafted ovary contained ripe follicles.

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CHOLIN IN THE BLOOD AFTER PARATHYREOIDECTOMY. BY W. F. SHANKS.

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W. F. KOCH(1) found in the urine of parathyreoidectomised dogs a number of toxic bases, including guanidin and dimethyl-guanidin, to which he ascribed the symptoms. Noël Paton, Findlay and Watson, in conjunction with Burns and Sharpe(2) independently arrived at the conclusion that guanidin or its methyl derivatives are the toxic agents in tetania parathyreopriva and in idiopathic tetany. Biedl(3, p. 292) in a résumé of the work upon the subject, concludes that the parathyreopriva *tetany is a guanidin intoxication.*

The source of the guanidin has, however, never been ascertained. Tetany is associated very frequently with rickets, and Noël Paton(4) has drawn attention to the possible importance of the disintegration products of lecithin in the etiology of the latter disease. One of the constituents of the lecithin molecule is of course cholin and if this be a source of methyl-guanidin the connection of tetany with rickets may be, in part at least, explained.

There is some evidence in support of the view that cholin may be converted into creatin (methyl-guanidin-acetic acid). Riesser(5) found that the creatin content of muscle increased after subcutaneous injection of cholin and also that there was an increased creatin excretion in the urine. He put forward the theory that a linkage took place between cholin and urea to form creatin. Baumann and his co-workers(6) performed numerous perfusion experiments with various substances in attempts to ascertain the precursors of creatin. Their results pointed to the possibility of cholin and urea and also of cyanamide acting as such. The last named, however, was so toxic as to place grave obstacles in the way of its experimental employment. Shanks(7) by intravenous administration of cholin obtained some evidence in favour of Riesser's views.

It is not difficult to imagine the formation of methyl-guanidin from creatin by oxidation of the acetic acid. Burns(8) found a steady increase of guanidin in the hen's egg during incubation (interrupted by a short

fall after the twelfth day), and recently Sharpe(9) has investigated the cholin content of eggs and has noticed a steady decrease as incubation advances.

Cholin as such does not circulate in any appreciable amount in the blood of the intact animal. Reid Hunt(10) noticed the extremely rapid disappearance of injected cholin and this has been fully confirmed by myself(11). There is no doubt that it is excreted only in small quantities in the urine. The great majority of workers have not succeeded in finding more than a very small percentage in the urine after administration by various routes (see Shanks(12)).

The evidence cited above of the possible conversion of cholin into creatin (and hence hypothetically into methyl-guanidin) suggested the performance of some experiments with a view to determining whether there was any increase of cholin in the blood of parathyreoidectomised animals. The material was obtained from animals parathyreoidectomised by Professor D. Noël Paton and Mr A. Watson.

Method. Blood samples were withdrawn from the animal before the operation and again when the symptoms were well developed. The blood having clotted, a measured quantity (usually 5 c.c.) of serum was placed in a parchment dialysing sac and dialysed for 24 hours against ten times its bulk of water. The dialysate was evaporated to dryness on the steam bath and extracted with absolute alcohol. The alcoholic extract was dried and any cholin present converted into the very active acetyl derivative by means of acetyl chloride. The acetylated products were tested in suitable dilutions on the excised heart of the frog. I have to thank Mr J. S. Sharpe for carrying out the initial steps of the separation of cholin in several cases.

From a considerable experience of such methods I can say that blood so treated is very satisfactory to work with. The residue for acetylisation is very small and if the acetyl chloride is carefully driven off the product dissolved in Ringer is almost perfectly neutral. Thus the necessity for addition of alkali to neutralise acid decomposition products of the acetyl chloride is avoided. The method precludes the possibility of the effects obtained being due to the deficiency of calcium in parathyreoidectomised serum described by Trendelenburg and Goebel(13).

Results. The results obtained show some variation but in several instances the serum extract after parathyreoidectomy was much more active in inhibiting the frog's heart, *i.e.* it contained more acetyl-cholin and consequently more cholin than normal serum extract. Details of some of the cases with slight and with marked symptoms are appended.

Exp.		Symptoms	Activity of serum extract compared with pre-operative sample from same animal
1	Dog	Moderate	10-fold more active
2	,,	Marked	Much more active
3	,,	Very slight	Slightly more active
4	Cat	Slight	Much more active
5	,,	,,	,, ,,
6	Dog	Marked	100-fold more active (see Fig 1)
7	,,	,,	2-fold more active

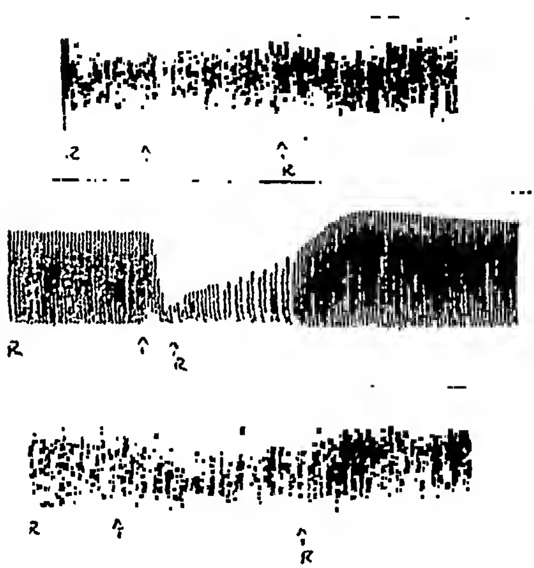

Fig. 1. Frog heart tracing. Perfused with serum extract at the arrow and with Ringer's fluid at *R*. *A*, normal serum extract. *B* and *C*, serum extract from parathyreoidectomised dog, *B* diluted ten times, *C* diluted 100 times.

Conclusion.

After parathyreoidectomy the serum in cases where the symptoms are well developed contains a larger amount of cholin than does normal serum from the same animal before operation.

The author would like to record his indebtedness to Professor Noël Paton at whose instigation the work was undertaken and who, throughout, took the keenest interest in it.

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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE RECOVERY PROCESS IN MUSCLE.

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It has long been known that oxidations analogous to those which are supposed to occur in living tissues are considerably influenced by the hydrogen-ion concentration. According to Mathews and Walker(1), the oxidation of cystein to cystin has an optimum hydrogen-ion concentration of about 10^{-8}, being reduced in speed on either side of this, but appreciably more on the acid side than on the alkaline. The following figures taken from their curve illustrate this fact:

*c*H	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Relative velocity	0·31	0·51	0·69	1·0	0·82	0·73

Hopkins' glutathione(2), on the acid side of neutrality, acts indeed as a hydrogen acceptor, thereby allowing a small amount of oxidation to occur: it does not, however, readily part with that hydrogen again and so is unable to work as effectively as a hydrogen transporter, and so to katalyse oxidations, as it can do on the alkaline side of neutrality. At a hydrogen-ion concentration of $10^{-7\cdot5}$ its effectiveness in katalysing oxidation is at an optimum, while at still lower hydrogen-ion concentrations its effect falls off again. Dixon and Tunnicliffe(3) found that the velocity of reduction of methylene blue by reduced glutathione is at its maximum at a hydrogen-ion concentration of about $10^{-7\cdot5}$, the relative velocities being, approximately,

*c*H	$10^{-7\cdot5}$	10^{-7}	10^{-6}	10^{-5}
Relative velocity	1·0	0·6	0·3	0·1

while the autoxidation of the sulphydryl compound has the following relative velocities

*c*H	10^{-5}	10^{-6}	$10^{-6\cdot5}$	$10^{-7\cdot3}$	10^{-8}	10^{-9}	10^{-10}
Relative velocity	0·04	0·24	0·41	1·0	0·8	0·7	0·65

Thus a rise of hydrogen-ion concentration is much more effective in diminishing the velocity of autoxidation than a corresponding fall.

[1] Working for the Medical Research Council.

The effect of hydrogen-ion concentration on the velocity of a biological oxidation in a living tissue could obviously be investigated by a study of the recovery heat-production of stimulated surviving muscle. Improvements in myothermic technique having made it possible (4) to follow, with fair precision, the actual time-course of the recovery process after moderate stimulation, it was possible to subject the same muscle in turn to various hydrogen-ion concentrations and to study their effect on the course of the recovery heat-production. There was a further reason for doing this, viz. that recent experiments of Anrep and Cannan (5) on the heart-lung preparation had shown that an alkaline condition of the perfusing blood tends to lead to the appearance of lactic acid in it, while an acid condition tends to its disappearance: it was possible that in the heart muscle the optimal hydrogen-ion concentration for the oxidative removal of lactic acid might be so far on the acid side (*a*) that an increase in the *c*H might tend to bring the process nearer to its optimal "reaction" and so to speed up the removal of lactic acid formed during exercise, and (*b*) that a similar decrease of *c*H might work in the opposite direction and so lead to delay in the removal of the acid.

Methods. There appeared to be two ways in which the hydrogen-ion concentration of the muscle might be varied, (*A*) to subject it to various concentrations of CO_2 in oxygen, and (*B*) to immerse it in a buffered Ringer's solution of known hydrogen-ion concentration. The former is only roughly quantitative, in respect of the absolute value of the hydrogen-ion concentration to which the muscle is subjected, but proved to give the more consistent and reliable results. In (*A*) in three successive sets of observations the oxygen in the muscle chamber was diluted (i) with a known percentage of N_2, (ii) with the same percentage of CO_2, and (iii) with the same percentage of N_2 again. In this way the effect of CO_2 could be ascertained directly by comparison, by altering the CO_2 percentage without varying the oxygen pressure; while a final return to the CO_2-free mixture enabled one to be sure that the effect was reversible and not the result of some permanent damage produced by the CO_2. In (*B*) two salt solutions very similar to those employed by Mines (6), but more effectively buffered and containing less acid and alkali, were made up as follows:

1 litre	Boric acid *M*/10	With either
1 „	Sodium acetate *M*/10	(1) 1 litre HCl *M*/10
200 c.c.	$CaCl_2$ *M*/10	or
300 „	KCl *M*/10	(2) 1 litre NaOH *M*/10
6·5 litres	NaCl *M*/8	

These were mixed in any proportion required to produce a given hydrogen-ion concentration, which was determined by a series of indicators and standards prepared by the British Drug Houses. Usually the muscle was left for half-an-hour in its chamber, in neutral or alkaline solution, the solution was then blown out by oxygen from a cylinder, and a few observations of the recovery heat-production made as soon as possible. The solution was then changed for another, of any required hydrogen-ion concentration: the muscle was left in this also for half-an-hour, and then again a few observations of the recovery heat-production made in oxygen. Finally a return was made to the first solution for half-an-hour, and again a few observations recorded in oxygen.

It should be noted that the effect on the recovery heat-production, of time elapsed from the start of the experiment, is of the same nature as the effect of an acid medium, since it has been found that, after any set of observations continued at intervals over a considerable period, the maximum rate of recovery becomes reduced and the whole process is spread out longer.

In Case (*A*) (*i.e.* CO_2) in three out of sixteen experiments the final curves of heat rate during the recovery process showed about as much departure from the original curves as might be expected from the time elapsed, for a muscle kept throughout in oxygen. In the other thirteen experiments the departure from the original was *less*; this can be accounted for by the effect of washing with Ringer's solution after CO_2 had been used, which tends to diminish the time effect to some extent: thus it may safely be assumed that the effect of CO_2, at least when not used in a strength of more than about 25 p.c., is completely reversible.

In Case (*B*) (*i.e.* buffered Ringer's solution) the effect of previous soaking in an acid solution always remained after soaking for half-an-hour in a neutral or even in an alkaline solution. The first record taken after soaking in the final alkaline solution was frequently very similar to that after soaking previously in an acid solution; successive records, however, taken while the muscle remained in oxygen, showed a tendency to return to the type of those taken after the original alkaline solution; this tendency to return was distinctly greater after the muscle had remained for an hour or so in oxygen. Thus, in Case (*B*), it is impossible to obtain a "reverse" and so properly to eliminate the time effect; any numerical result for showing the effect of increased *c*H can be obtained only by comparing directly the observations (1) after soaking in neutral or alkaline solution, (2) after soaking in acid solution. The results therefore

will not be so reliable as those obtained by the use of CO_2: the general effect, however, of the acid solution is as shown in Fig. 2.

Results. In no case was it observed that an alteration of the hydrogen-ion concentration had any effect on the total recovery heat-production: the latter remains consistently about 1·5 times the total initial heat. presumably therefore the nature and completeness of the recovery process are unaffected by the hydrogen-ion concentration. The velocity, however, with which it occurs is largely affected, as is shown by the curves of Fig. 1. Here we see that 15 p.c. of CO_2 in oxygen diminishes the velocity

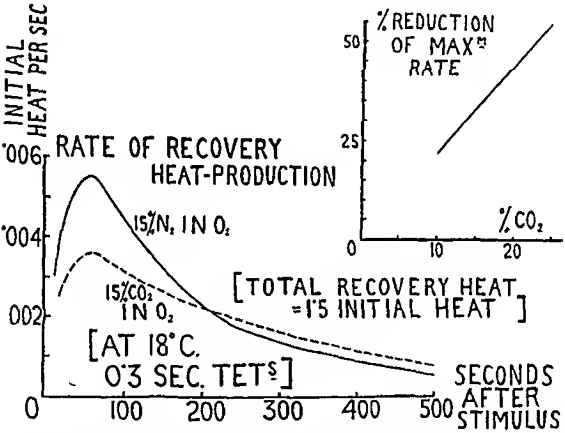

Fig. 1. Effect of CO_2 on the time-course of the recovery heat-production. Inset, the effect of varying percentages of CO_2 on the maximum rate of heat-production during recovery.

of the recovery process in its early stages by about 33 p.c.; naturally, of course, if the total extent of the recovery process remains the same, this lower rate at the start must be compensated by a higher rate later on, as indeed we see. The best measure of the vigour of the recovery process is afforded by its maximum rate, a quantity which is fairly accurately obtainable in the analysis of the records, and occurs sufficiently early to make it a reasonable criterion of the speed of recovery. If the percentage diminution in the maximum rate of recovery heat-production be plotted against the CO_2 pressure, the result is a straight line, as shown

in the diagram inset in Fig. 1. The speed, therefore, of the oxidative process of recovery diminishes as a linear function of the CO_2 pressure.

The CO_2 contained in muscle, in combination as bicarbonate, under ordinary conditions, has been determined by Meyerhof(7). It amounts to about 0·1 c.c. per gm. of muscle. If this quantity of combined CO_2 were constant, and independent of the CO_2 pressure, the hydrogen-ion concentration of the muscle could be calculated directly by Henderson's equation from the CO_2 pressure and the volume of combined CO_2. Assuming for the constant k in the formula,

$$cH = k . pCO_2/vCO_2,$$

the value found by Barcroft(8) and his collaborators for human blood, viz. $4{\cdot}7 \times 10^{-8}$, the hydrogen-ion concentration of the muscle may be calculated as follows:

p.c. of CO_2	10	20	30	40	50
cH	$10^{-6\cdot5}$	$10^{-6\cdot2}$	$10^{-6\cdot0}$	$10^{-5\cdot9}$	$10^{-5\cdot8}$

This calculation is admittedly rough: and since it is probable that muscle, like blood, increases its content of combined CO_2 when subjected to increasing CO_2 pressure, the calculated cH's are probably too high at the higher CO_2 pressures. They should, however, certainly be of the right order of quantities. Thus an increase of the cH from the normal value of the resting frog's muscle to something less than $10^{-5\cdot8}$ is sufficient to diminish the speed of the recovery heat-production by some 50 p.c. This effect is comparable in magnitude with that of an increase of cH on the velocity of autoxidation of cystein or glutathione: it suggests that the normal oxidations occurring in muscle are similar in nature to these known types.

It is not, of course, certain that the active portions of the muscle have their cH as much affected by CO_2 as the calculation suggests: though it is certain that they must be freely permeable to CO_2, since the latter is formed in oxidation and has to escape. The work moreover of Jacobs(11, 12), referred to below, makes it very likely that the presence of CO_2 outside rapidly affects the cH inside the fibre. The possible increase of the combined CO_2 of muscle, with increase of CO_2 pressure, would seem to be worthy of a special investigation, both in this connection and in that of the buffers of muscle: it appeared undesirable, however, to hold up the publication of our present results until such an investigation had been made. The true hydrogen-ion concentration of the muscle cannot well be greater than that calculated as above, so that the effect

observed on the recovery process is due to a comparatively small change of *c*H.

The effects found with the huffered Ringer's solution may be divided into two categories: (*a*) those obtained on the alkaline side, and (*b*) those on the acid side of neutrality. Fifteen good experiments were performed, all at 15° C., the muscle being stimulated in oxygen after soaking in the solution for half-an-hour. Of these, six experiments were on the alkaline side, and made it certain that there is no appreciable change in the speed of the recovery process between hydrogen-ion concentrations of 10^{-7} and $10^{-10 \cdot 2}$. In one of these experiments four successive records after exposure to a *c*H of 10^{-7}, four after exposure to a *c*H of $10^{-10 \cdot 2}$, and four after returning to a *c*H of 10^{-7}, were practically identical. On the acid side, however, a difference immediately became obvious. A change from 10^{-7} to $10^{-6 \cdot 5}$ produced an observable

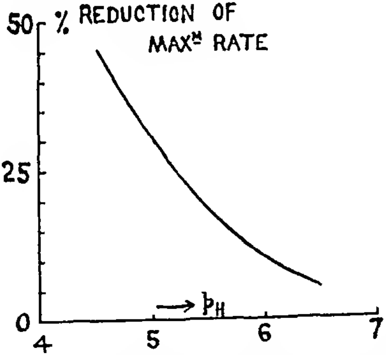

Fig. 2. Effect of hydrogen-ion concentration of the fluid in which a muscle is immersed on the maximum rate of heat-production during recovery.

effect, and a higher cH had a geater effect, as shown in the diagram (Fig. 2). Here the effect is measured by the percentage drop in the maximum recovery rate when compared with that occurring at neutrality. At hydrogen-ion concentrations greater than $10^{-4 \cdot 5}$ the initial deflection due to the heat-production usually fell off, and the movement of the thermopile zero indicated a continued small production of heat, in a manner making good records unobtainable. This is presnmably due to

a harmful effect of such high *c*H's on the muscle itself, causing the spontaneous liberation of lactic acid in it.

The numerical results obtained with the buffered Ringer's solution are not very consistent in the different experiments. This is due, at least in part, as indicated above, to the impossibility, in this case, of obtaining an accurate "reverse," *i.e.* a return to the initial alkaline or neutral condition, after subjection to acid. In the case of the CO_2 experiments an accurate "reverse" was obtained, so we regard those experiments as being the more reliable. Apparently the effect due to an acid buffered Ringer's solution takes a long time to pass away. Thus the curve in Fig. 2 cannot pretend to be as accurate as that of Fig. 1, but it gives the mean results of all the experiments in acid and certainly shows the general effect of a high *c*H.

We see therefore that there is general agreement between the two methods, in the conclusion that a rise in hydrogen-ion concentration considerably diminishes the speed of the recovery process. The CO_2-effect therefore is not altogether a specific one, but is due (in part at any rate) to the rise of *c*H caused by it. On the other hand, the effect of a change of *c*H produced by the buffered Ringer's solution would appear to be considerably less than that caused by CO_2, if the calculation given above for the *c*H of muscles at different CO_2 pressures be anywhere near the truth. It would seem probable that CO_2 is able freely and rapidly to permeate the muscle fibre, and so to cause its consequent rise of *c*H fairly soon: but that the acids present in the buffered Ringer's solutions take a very long time to pass into the muscle, and so do not (in half-an-hour) produce their full change of *c*H inside the fibre. This is borne out by the fact that a change in the speed of the recovery process produced by subjection to acid conditions is not readily reversed by a return to a neutral or alkaline medium. Only after very prolonged soaking in a neutral or alkaline solution can the effect of a previous acid solution be abolished. It would seem likely that the effect of an external change in the hydrogen-ion concentration, on the recovery heat-production, is usually diminished by the fact that an equilibrium takes so long to be attained between the external solution and the inside of the fibre. The true effect, therefore, of a change of *c*H inside the muscle itself is more correctly shown by the experiments in CO_2, which is able freely to pass in and to affect the *c*H inside: in these experiments calculation from Fig. 1 shows that a comparatively small change in hydrogen-ion concentration produces a relatively large diminution in the speed of the recovery process, an effect analogous to, and comparable in magnitude

with, that found to occur in autoxidations similar to those probably occurring in the muscle.

That the difference between the effects of CO_2, and of buffered Ringer's solutions respectively, is due to a difference in the permeability of the cell wall by carbonic and other acids, is strongly supported by the work of Jacobs (11, 12). CO_2 in solution was shown to be enormously more effective, in killing tadpoles or protozoa, or in exciting the taste receptors of the human tongue, than other acids in solution at the same hydrogen-ion concentration. Even in the presence of sufficient bicarbonate to render the solution alkaline, the specific effect of CO_2 still occurred. This effect might have been attributed to some molecular character of CO_2 as such, rather than to its facility in passing through the cell envelope and so producing a change of cH inside. That the latter however is the true explanation is shown by further experiments on the flowers of *Symphytum peregrinum*, which contain an indicator of hydrogen ions in their pigment, which is pink at higher cH's, blue at lower. All acids, other than CO_2, affect the colour of the pigment very slowly, while CO_2 can produce an acid effect inside, even when dissolved in an alkaline bicarbonate solution outside. A solution of CO_2 in bicarbonate produced just as vivid an effect on the colour of the indicator in the flower as water with 4000 times the hydrogen-ion concentration. It would seem that CO_2 has specific powers of penetrating living cells, but that, once inside them, it produces its effect merely by altering their hydrogen-ion concentration.

These autoxidations are known to have an optimum at, or somewhat near, neutrality: they diminish in speed on the alkaline, as well as on the acid side, though much more noticeably on the latter. The recovery process in muscle appears to be absolutely uninfluenced by making the reaction more alkaline than neutrality. It is conceivable that this may be due to the muscle being impenetrable to the alkalies present in our buffered Ringer's solution, and unfortunately it is not possible to use the CO_2 method on the alkaline side. The complete absence, however, of any effect on the alkaline side, when compared with the immediate effect observed on the acid side, suggests that the result obtained is a genuine one, and that the recovery process approaches its maximum speed asymptotically as the cH is diminished, attaining that value finally at, or about, neutrality. If so, in this respect the recovery process is unlike the autoxidation of cystein or glutathione: but analogous rather to the phenomenon observed by Hartridge and Roughton (9), who found the velocity of the reaction

$$HbO_2 \rightarrow Hb + O_2$$

to vary with the hydrogen-ion concentration, being low and constant, at, and at less than, one of $10^{-7.7}$, high and constant, at, and at more than, one of $10^{-6.3}$, rising gradually from one level to the other between these limits. In this case, as suggested by Brown and Hill(10), the diminished affinity of hæmoglobin for oxygen, as the hydrogen-ion concentration increases, is probably an ionic effect, the ion having many times the affinity for oxygen that the undissociated molecule has. As the hæmoglobin solution passes from a cH of $10^{-7.7}$ to one of $10^{-6.3}$, the hæmoglobin —originally fully dissociated into its ions—becomes gradually less dissociated, and finally completely undissociated. It would seem possible that the effect of a rise of hydrogen-ion concentration on the recovery process involves a similar ionic mechanism, the velocity of oxidation being dependent on the presence of some ionised bodies in the muscle, being diminished in speed therefore by a reduction in their degree of ionisation. An analogous suggestion was made by Mathews and Walker(1) to explain the existence of an optimal reaction for the oxidation of cystein, viz. that the neutral, non-ionised molecule in this case oxidises the most rapidly.

Finally, it is obvious that the results of Anrep and Cannan(5) cannot be explained on the basis of our experiments. A rise of cH diminishes, under all circumstances, at any rate in frog's skeletal muscle, the speed of the recovery process, and so would not tend to accelerate the oxidative removal of lactic acid introduced into the blood perfusing a heart-lung preparation: nor does making the reaction more alkaline diminish the speed of the recovery process, as would be required to explain the appearance of lactic acid in the blood made alkaline by over-ventilation. Clearly the mechanism of their results is of a completely different character.

SUMMARY.

1. The effect of a rise of hydrogen-ion concentration is to diminish, in frog's skeletal muscle, the speed of the oxidative recovery process, as measured by the recovery heat-production. This is true whether the rise of hydrogen-ion concentration be produced by exposure to CO_2, or by buffered Ringer's solution.

2. The effect of CO_2 is rapid and completely reversible, showing that the CO_2 readily permeates the muscle fibre. The effect obtained with buffered Ringer's solutions of different hydrogen-ion concentrations is *not* so readily reversible, although a return is possible after very prolonged subjection to the initial solution: which suggests that the acids in a

buffered Ringer's solution do not freely penetrate the muscle. This is in keeping with the findings of Jacobs, on the specific penetration of cells by CO_2.

3. A rough calculation of the hydrogen-ion concentrations produced in muscle by various pressures of CO_2, shows that the effect of an increase of hydrogen-ion concentration on the speed of the recovery heat-production in muscle is comparable with its effect on the autoxidation of cystein or glutathione.

4. A fall, however, of hydrogen-ion concentration in the fluid bathing a muscle, from neutrality to $10^{-10 \cdot 2}$, has no effect on the speed of the recovery process: differing in this respect from its action in the case of cystein or glutathione.

5. It is possible that the mechanism of oxidation in the recovery process is analogous to that of the autoxidation of such bodies as cystein, or of reactions katalysed by the presence of a hydrogen transporter such as glutathione. If so, the absence of any effect of increased alkalinity must be explained by the hypothesis that alkalies in solution do not penetrate the muscle fibre. It is possible, however, that the recovery process is dependent upon the presence of some ionised intermediary or katalyst, whose ionic dissociation is complete at, and beyond, neutrality, but diminishes, like that of a weak acid, as the hydrogen-ion concentration rises.

The expenses of this research were defrayed in part by a grant from the Royal Society.

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THE INNERVATION OF THE PYLORIC SPHINCTER OF THE RAT. BY M. NAKANISHI.

(*From the Physiological Laboratory, Cambridge.*)

WHILST numerous observations have been made on the behaviour of the pyloric region of the stomach, few have been made on the effect of nerve stimulation on the pyloric sphincter as distinguished from the rest of the pyloric region. Openchowski (1), in experiments in which some part of the central nervous system was stimulated, came to the conclusion that the thoracic nerves as far as the 10th sent inhibitory and motor fibres to the pyloric region inclusive of the sphincter: the inhibitory fibres being more numerous than the motor in the dog and less numerous than the motor in the rabbit. Elliott (2) briefly mentions that the pyloric sphincter of the rabbit is contracted by adrenaline and also by stimulation of the splanchnic nerve, but that the contraction is weak. Smith (3) in the course of observations on strips of the stomach removed from the body found that adrenaline caused slight contraction of the pyloric sphincter in a few instances, but that in most cases no reaction could be obtained.

I have investigated the behaviour of the sphincter in rats by the following method. The rats were anæsthetised with urethane and kept warm by a bag containing hot water. A small glass tube was introduced into the stomach through the œsophagus and tied in the œsophagus. The tube was connected with Mariotte's bottle containing warm Ringer's solution. The right side of the abdomen was incised and another larger glass was inserted into the duodenum for recording the flow of fluid from the stomach by a drop-recorder.

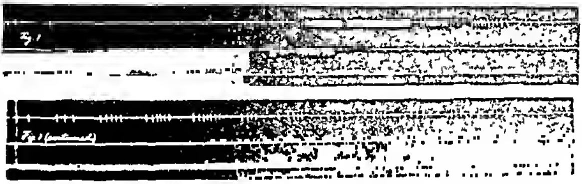
The critical intragastric pressure which was sufficient to force fluid through the sphincter was at first about 11–15 cm. water pressure. It varied during the experiment and tended to become higher in the case of the vagus stimulation. The observations were begun a few centimetres below the critical pressure, the sphincter then generally had periodic openings.

For stimulation of the nerves I used induction shocks of such strength as to be felt fairly strongly on the tip of the tongue. Sometimes I used also a stimulus which was only felt feebly.

The vagus. The peripheral end of the vagus was stimulated (*a*) on the œsophagus a little below the diaphragm, (*b*) in the neck after giving atropine, (c) in the neck without giving atropine.

In stimulating the nerve below the diaphragm the branch on the ventral surface of the œsophagus was taken. With a fairly strong stimulus, relaxation of the sphincter was constantly obtained. The degree of relaxation and its duration varied considerably in different experiments, and in any one experiment varied with successive stimuli.

Fig. 1 is an example of marked relaxation when there is high initial tone; the cessation of the stimulus is followed by rhythmic contraction and



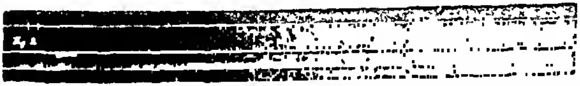


Fig. 1. Marked relaxation of the pyloric sphincter caused by fairly strong stimulation of the anterior vagus branch below the diaphragm. Upper line: drops of fluid from the stomach. Middle line: stimulation of the nerve. Under line: time in seconds.

Fig. 2. Contraction of the pyloric sphincter caused by weak stimulation of the anterior vagus branch below the diaphragm.

relaxation, the duration of the relaxation gradually decreasing. Weaker stimulation in all cases but one gave a similar but less effect. In the exception, there was contraction of the sphincter instead of relaxation (Fig. 2) showing the presence of motor as well as of inhibitory fibres in the vagus. In some cases, especially when the tone of the sphincter was low, and fluid was passing at short intervals through it, the primary increase of flow caused by stimulation was followed by a decrease, indicating the presence of motor fibres: this however was not a marked feature of the results. The greater the tone, the longer was the latent period and the more obvious the effect of vagus stimulation. Figs. 3*a*, 3*b* show the effect of two stimuli of equal strength, the former at the beginning

of an experiment with low tone, the latter after numerous stimulations when the tone had increased. (Time in seconds in all figures.)

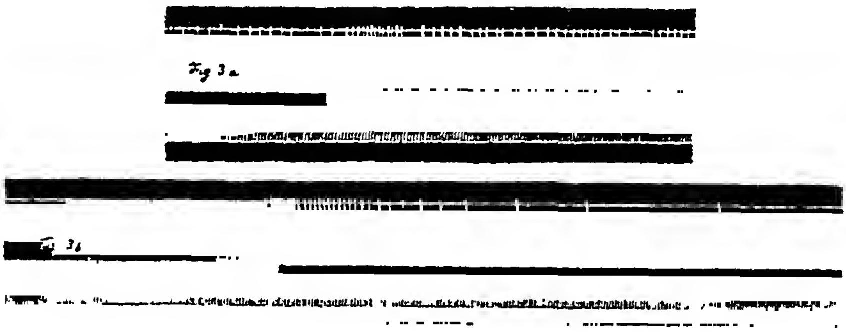


Fig. 3. Relaxation of the pyloric sphincter caused by weak stimulation of the anterior vagus branch below the diaphragm: *a*, at the beginning of an experiment with low tone of the sphincter:—relatively short latent period before the relaxation: *b*, when the tone of the sphincter had increased after repeated stimulation of the nerve:—long latent period and greater, though less lasting, relaxation.

When atropine (1·5 mgrm.) was injected into the femoral vein and the peripheral end of the cervical vagus stimulated, the results were much the same as those described above. So far as the experiments went, the after-contraction of the sphincter was greater (Fig. 4) with fairly strong stimulation. Weaker stimulation once, but only once, caused trifling

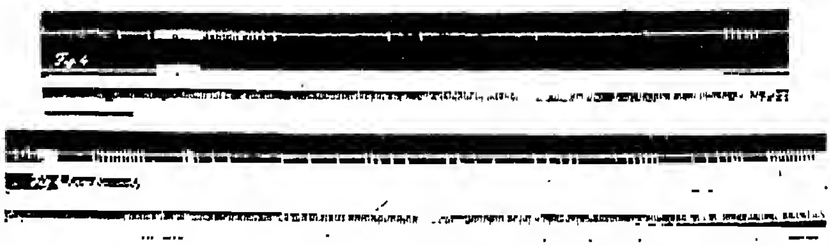


Fig. 4. Relaxation and after-contraction of the sphincter caused by fairly strong stimulation of the vagus nerve in the neck after atropine injection.

preliminary contraction. Stimulation of the vagus in the neck when atropine was not injected gave results not distinguishable with certainty from those obtained after atropine injection.

The splanchnic nerve. The left splanchnic nerve was exposed and cut in the abdomen. Since the nerve is small in the rat, it was taken with a little fatty tissue surrounding it. Stimulation of the nerve caused con-

traction of the sphincter (Fig. 5). The effects were not due to setting free of adrenaline, for contraction was obtained by stimulating the

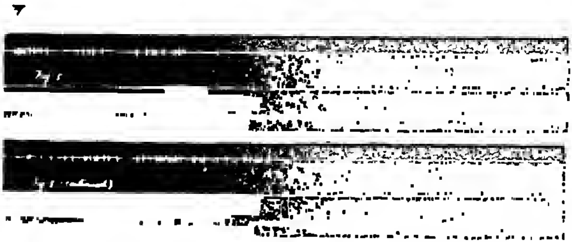


Fig. 5. Contraction and after-relaxation of the sphincter caused by stimulating the left splanchnic nerve.

splanchnic nerve after extirpation of the suprarenal glands. The contraction was usually followed by relaxation; this varied in character, sometimes being irregularly rhythmic, sometimes being fairly regular and slight. It may be mentioned that in one case the splanchnic was mechanically stimulated by traction and this had the same effect as electrical stimulation.

The preceding results show that in the rat stimulation of the vagus has usually a predominant inhibitory effect and the splanchnic usually a predominant motor effect. The effect of adrenaline was then tried.

Effect of adrenaline. In the first place this was applied locally. A small piece of cotton wool soaked in 0·1 p.c. adrenaline was placed on the outer surface of the pyloric sphincter. The effect was like that produced by

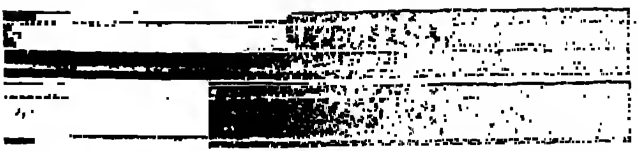


Fig. 6. Contraction of the pyloric sphincter caused by local application of adrenaline. Middle line: placing and taking off of the cotton-wool soaked in adrenaline.

Fig. 7. Contraction of the sphincter caused by intravenous injection of adrenaline. ×, injection of adrenaline.

stimulating the splanchnic nerve, but the contraction, though it took some time to produce, was greater (Fig. 6). In the example given the

contraction was followed by rhythmic dilatation. Intravenous injection of adrenaline had a similar effect (Fig. 7).

SUMMARY.

The experiments show that the predominant effect of the vagus is inhibition of the pyloric sphincter and that of the sympathetic is contraction, *i.e.* the predominant effects of the two systems of nerves are antagonistic. But it is clear that the vagus has also some motor fibres for the sphincter; these, no doubt, are brought into play in vomiting. The evidence that the sympathetic contains some inhibitory fibres for the sphincter is less satisfactory; since inhibition on stimulating the splanchnic nerve was only obtained as a secondary effect after a preliminary contraction. Adrenaline, as in so many other cases, produces an effect broadly similar to that caused by sympathetic stimulation.

I am greatly indebted to Prof. Langley for his kind advice and suggestion during the course of my work.

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PROCEEDINGS

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The force exerted by contracted capillaries.

By THOMAS LEWIS[1].

In a previous communication Cotton, Slade and Lewis(1) have demonstrated that the "white line" produced on the surface of human skin by light stroking is due to an active contraction of the capillaries. The data upon which this conclusion was based have been fully confirmed, in part by Ebbecke(2), and in part by Carrier(3) who come to substantially the same conclusion as that at which we arrived.

I have now used this "white line" reaction in studying the force which actively contracted capillaries of the human skin can exert. The method is a simple one. A line (or band) of pallor is produced on the skin surface in a subject who shows the phenomenon vividly (this is usually a young subject), the white line is then investigated. Suction is applied by means of a glass capsule, and the pressure over the line reduced until it takes the tint of the neighbouring skin and becomes indistinguishable, or the venous pressure is raised in the corresponding tissue area, by constricting the veins at accurately known pressures, until again the line of pallor is abolished. Each method approximately indicates the amount of force required to distend the contracted capillaries. The first method is applicable to the skin of any part of the body, the second to the skin of the limbs only.

The following example is an illustration. In a young man (whose systolic B.P. is 115 mm. Hg) a band of skin pallor, produced by stroking either forearm, interscapular region or lower part of the calf, is just abolished by suction at pressures varying between −90 and −100 mm. Hg. Similar bands on the forearm are not materially changed by raising the venous pressure in the limb to 90, and are reduced, but not abolished, by raising it to 100 and 110 mm. Hg. The two distinct methods of testing the arm sufficiently confirm each other. From these observations it is to be concluded that a force, equivalent to or exceeding 100 mm. Hg

[1] Working on behalf of the Medical Research Council.

is needed to distend the actively contracted capillaries in this subject; a similar force is therefore exerted by the contracted capillaries on their contents. It is probable that the measures obtained are lower than those which the capillaries can exert, since capillary pressure necessarily exceeds venous pressure.

The magnitude of the force, as measured in other individuals, is usually less than that ascertained in the above illustration, ranging, so far as present experience shows, from 50 to 100 mm. Hg, and does not usually approach so closely to the systolic arterial B.P. as in this case.

These observations help to explain why capillary distension in the human foot does not occur on assuming the erect posture; contractility of the capillary wall in the foot would have little functional value if it were not highly developed, and unless the capillaries of the foot were not normally tonically contracted. Appreciable or constant differences, in the power of the capillaries of various skin areas to exert pressure on their contents, have not been found.

If the capillaries are first dilated by suction or by venous congestion, the pressure which they are then able to exert on their contents, as shown by the subsequent development or non-development of the "white line," is naturally found to be less, sometimes much less, than is indicated by the values here given.

The white reaction may also be produced by lightly stretching the skin; it is suggested that the reaction comes into play in conditions of exercise and that when, for example, a limb is moved, the blood tends to be diverted from the skin vessels to the vessels of the muscles.

Note. The term capillaries, as used in this communication, is a brief equivalent for those minute vessels which are responsible for skin colour.

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A film method for the reaction of the liquids of the body by indicators. By G. A. BUCKMASTER.

Wire rings or loops varying in size and made of various metals were on trial found to be useless for making durable films. However with suitably constructed celloidin rings, films, which are practically colourless, of serum, bile, urine and other liquids remain unbroken for a long time, often for an hour.

The indicator is added from a pipette, constructed as a stalagmometer which delivers a drop of constant size, to two films; one of these is the liquid to be examined, the other a phosphate-citric acid buffer standard solution[1].

The colours can be exactly matched by reflected light and the value of the P_H of the standard employed is read off directly from a graph.

Purkinje fibres in the auricles of birds[2]. By A. H. HOLMES. (*Preliminary Communication.*)

The heart of the following birds has been investigated for the presence and distribution of Purkinje fibres: ostrich, rhea, pigeon, duckling, gosling, fowl, swan, linnet, cormorant, toucan. Sections have been cut 8 or 10 microns in thickness, and stained by hæmatoxylin and eosin, or by hæmatoxylin and Van Gieson's stain. In this communication, reference will only be made to the findings in the auricles; these may be expressed in the form of a table.

	Endoc. of r. aur.	Myoc. of r. aur.	Epic. of r. aur.	Auric. septum	Right venous valve	Left venous valve	Endoc. of l. aur.	Myoc. of l. aur.	Sulc. term
Ostrich	+	+	+	+	+	+	+	+	+
Rhea	+	+	+	0	.	.	+	+	0
Pigeon	+	0	0	+	.	.	+	+	.
Swan	+	0	0	+	.	.	+	+	.
Cormorant	+	+	0	0	.	.	+	+	.
Duckling	+	+	0	.	.	.	+	+	.
Gosling	+	0	0	0	.	.	+	+	.
Fowl	0	0	0	0	.	.	0	0	.
Linnet	0	0	0	0	.	.	+	+	.
Toucan	0	0	0	+	.	.	0	0	.

Purkinje fibres are recognisable by their size and staining. Usually they are larger in cross section than the ordinary myocardial fibres, and they always stain less deeply than the latter, on account of their incomplete fibrillation. In the ostrich a Purkinje fibre may be 20 times the diameter of a myocardial fibre, and the contrast in staining may be very marked. In other birds, such as the duck and the fowl, the contrast as regards both size and staining may be very slight indeed, and only recognisable under high magnification. Purkinje fibres may be seen in direct continuity with myocardial fibres; in the ostrich their gradual reduction in size to that of myocardial fibres is readily observed. Every-

[1] McIlvaine. *Journ. of Biol. Chem.* 49. p. 183. 1921.

[2] Observations undertaken for the Medical Research Council.

where Purkinje fibres appear closely associated with connective tissue, whether this be under the endocardium, around arteries in the myocardium, or in the epicardium.

No association with nerves has been observed in this investigation. No special strands of Purkinje fibres have been found such as would suggest paths of stimulus conduction, though the fibres are most plentiful under the endocardium covering the larger trabeculæ. Although tissue resembling the sino-auricular node of mammals was found near the orifice of the superior vena cava of the ostrich, nothing corresponding to the auriculo-ventricular node was found in any heart.

The present investigation of the differentiation, amount, and distribution of Purkinje fibres suggests that these fibres do not form the path of conduction of the stimulus to contraction in the auricles of birds.

Thermodynamical proof of the reciprocal relationship of oxygen and carbon dioxide in blood. By GILBERT S. ADAIR.

Consider the equilibrium of four components:

$$Hb + O_2 = HbO_2 + z\,CO_2 \quad \text{.....................(1).}$$

z = mols CO_2 expelled per mol Hb oxidized.

If the reaction followed the law of constant proportions, equilibrium would be given by an equation of the form of Gibbs (121). [Equilibrium of Heterogeneous Substances.]

$$\mu Hb + \mu O_2 = \mu HbO_2 + \mu CO_2 \quad \text{.................(2),}$$

where μHb is the molecular thermodynamic potential of hæmoglobin. Since z is a function of $[CO_2]$ the differential of (2) must be applied:

$$\mu Hb\, d\,[Hb] + \mu O_2 d\,[O_2] = \mu HbO_2 d\,[HbO_2] + \mu CO_2 \,.\, z \,.\, d\,[CO_2].$$

Let the concentrations of Hb and HbO_2 be constant.

Let $\mu Hb - \mu HbO_2 = \text{constant} = k$.

$$\therefore\ k + \mu O_2 d\,[O_2] = \mu CO_2 z d\,[CO_2].$$

Let z be constant over the short range of CO_2 concentrations $[CO_2]_1$, $[CO_2]_2$.

Let
$$\mu O_2 \ = \text{const.}\ k_1 + RT\log\,[O_2],$$
$$\mu CO_2 = \quad ,, \quad k_2 + RT\log\,[CO_2].$$

$$\therefore\ k + k_1 + RT\log\,[O_2]_1 = zk_2 + zRT\log\,[CO_2]_1,$$
$$k + k_1 + RT\log\,[O_2]_2 = zk_2 + zRT\log\,[CO_2]_2.$$

$$\therefore\ \log\,[O_2]_1 - \log\,[O_2]_2 = z\,(\log\,[CO_2]_1 - \log\,[CO_2]_2),$$

$$\therefore\ d\log\,[O_2]/d\log\,[CO_2] = z \quad \text{.................(3).}$$

Hence z, the CO_2 expelled on oxidation, can be calculated by measuring oxygen tensions and CO_2 tensions at a definite saturation, and conversely, the effect of CO_2 in increasing the oxygen pressure required to reach a given saturation can be calculated from measurements of z at different CO_2 tensions.

$$\log[O_2] = \int z d \log[CO_2] \quad \ldots\ldots\ldots\ldots\ldots\ldots (4).$$

Integrating (4) with the data of Christiansen, Douglas and Haldane, the calculated oxygen pressures at 80% HbO_2 are 23, 36, 45, 67 for CO_2 tensions 3, 20, 40, 80. The observed O_2 pressures on Barcroft's blood were 24·5, 35, 45, 62. The slight discrepancy is due to the separation into plasma and corpuscles. The beautiful simplicity of formulæ 3 and 4 is evident only in homogeneous solutions.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

November 17, 1923.

The behaviour of lecithine, hydrolecithine and cholesterol in monomolecular films. By J. B. LEATHES. (*Preliminary Note.*)

Lecithine forms a film on water and when studied by the improved technique of N. K. Adam this film is clearly an "expanded" film, the area reckoned for each fatty acid chain at 1·4 dynes per cm. for two preparations made in this laboratory by Levene's method being 55 and 58Å^2 at room temperature and for a third completely analysed specimen (for which I am greatly indebted to Dr Levene) 56·6 at 15°, 52·6 at 4·5°; even at this latter temperature there was no sign of condensation at 12 times this pressure.

But a preparation of hydrolecithine, also given me by Dr Levene, gives on the other hand a condensed film, in which at 1·4 dynes per cm. each fatty acid chain occupies the area 28 Å^2, and even under the very high compression that this substance withstands, of the order of 200 atmospheres, the area is not as small as that of palmitic at one-fortieth of this pressure.

So the presence of the phosphoric acid and choline in the part of the molecule that rests on the water prevents close packing of the paraffin chains in a condensed film. The expansion of lecithine films is due to the unsaturated acid radical; for oleic acid and triolein give expanded films, stearic and tristearin condensed ones, as Adam has shown.

Cholesterol gives a condensed film with molecular area 38 Å^2 at 1·4 dynes per cm.; at twenty times this compression only about 3 p.c. less; so the compressibility is small. If the specific gravity of cholesterol in the film is the same as that of the substance in bulk, if that is to say there is in this latter state no interlocking of the molecules in successive layers, the thickness of the film would be 17 Å, the compression force required to reduce the area of the film by 3 p.c. would be about 170 atmospheres and the vertical length of the molecule would be nearly three times its transverse diameter.

Since cholesterol palmitate does not give a monomolecular film it appears certain that it is the hydroxyl group in the cholesterol that binds the molecule to the water surface, reinforced it may be by the unsaturated pair of carbon atoms which Windaus has shown to be near by in the adjacent 5 ring.

Cholesterol mixed with lecithine or fatty acids in the same film tends to cause closer packing, detectable even with palmitic acid at ordinary temperature on dilute acid, but amounting to a reduction of area of 30 to 40 p.c. in the case of expanded films (palmitic acid at 35° to 40°, tridecylic acid at room T°); with lecithine the reduction of area when mixed with cholesterol was less than this but still definite: even with hydrolecithine it was also definite.

Experimental alterations in the oxygen and carbon dioxide tensions of air between the skin and the muscles. By J. ARGYLL CAMPBELL.

In previous observations[1] it was shown that injection of air between the skin and the muscles afforded a ready means to examine changes of carbon dioxide tension in tissue spaces under various conditions. In the present observations the oxygen tension was also estimated. Air was injected under the skin (rabbits) of the back and left there until the oxygen and carbon dioxide tensions therein had come into equilibrium with the tensions of these gases in the tissue spaces. In the case of oxygen this was found to have occurred in about thirty-six hours, the tension being at about 20 to 30 mm. On the other hand the carbon dioxide tension equilibrium was established in an hour or so, at about 40 to 50 mm. Hg.

Exercise. Two minutes after cessation of exercise to produce fatigue the carbon dioxide tension was markedly increased (by 15–20 mm. and more), but in an hour or so fell below the original level. The oxygen tension was usually slightly decreased at first, but later rose well above the original level.

Artificial respiration. Both the carbon dioxide and the oxygen tensions were decreased by vigorous artificial respiration, the former to a marked degree. Some time after cessation of the artificial respiration the tensions returned towards normal.

Pituitary extract. Subcutaneous injection of "Infundin" (1 c.c. per kilo) markedly increased the carbon dioxide tension (by 27 mm.); on the other hand the oxygen tension was markedly decreased (by 24 mm.). As the effect passed off the tensions returned to and beyond normal.

[1] *Journ. of Physiol.* 57. p. 275.

Adrenalin. Subcutaneous injection of Adrenalin chloride (·25 mg. per kilo) increased the carbon dioxide tension (by 15 mm.) and decreased the oxygen tension (by 12 mm.). After several hours the tensions returned to and beyond normal.

Urethane. Subcutaneous injection of the usual doses of urethane increased the carbon dioxide tension for the first few hours (by 15 mm.), but later the tension fell towards the normal level; on the other hand the oxygen tension fell (by 5 mm.) for the first few hours; and later rose again towards the original level although the animal was still deeply under the influence of the anæsthetic.

External temperature. My previous results[1] for the effect of changes of external temperature on the carbon dioxide tension were really due to urethane, since further observations on unanæsthetised animals have shown that marked changes of external temperature (1° C. to 37° C.) produced as a rule no appreciable changes in the tension of this gas nor in the tension of oxygen.

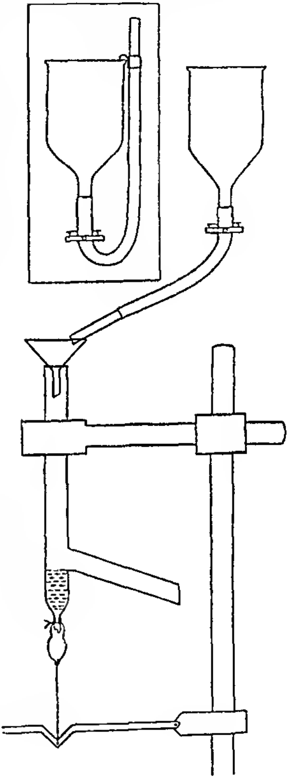
All the above variations may be explained by circulatory and metabolic changes.

On the action of alcohol. By R. J. S. McDowall.

It is usually stated that alcohol has little effect on the circulation apart from its dilating effect on the superficial vessels which is compensated by constriction of internal vessels. In animals under chloralose, injections of dilute alcohol intravenously cause a profound fall in venous pressure with, it may be, no change in arterial pressure. It is suggested that this fall of venous pressure and the consequent relief of the right side of the heart is the basis of the alcohol therapy so strongly adhered to by many clinicians in cases of cardiac embarrassment. Other facts indicate that the failure to recognise this fall hitherto may have been due to the use of anæsthetics which depress the circulation. The maintenance of the arterial pressure appears to depend to a large extent on the integrity of the vagus and experiments are being carried out to see if this is really a vago-pressor reflex, *i.e.* the opposite of the depressor reflex.

A constant pressure perfusion cannula. By W. F. Shanks.

This cannula is designed for perfusion of the excised heart of the frog and is tied into the vena cava. Its principle is obvious from the diagram. The perfusion fluid on reaching a certain level runs off through the side tube. By means of the screw clip on the supply tube from the reservoir it is a simple matter to adjust the flow to allow of this with a minimum of waste but the delivery of fluid to the heart is, within limits, independent of the clip. The apparatus is particularly suitable for testing the action of drugs. Its advantages are:



1. Maintenance of a constant head of pressure.
2. The dead space is very small and a test fluid acts on the heart almost instantly.
3. Tests can be carried out with very small quantities of fluid.
4. The open connection between cannula and reservoirs enables the latter to be manipulated at will without the least disturbance of the heart. Any number of reservoirs can be used.
5. Readjustment of the screwclips on the supply tubes can be obviated by hooking the latter up when not in use.

The apparatus has given satisfaction for class work at Glasgow University. It originated in a suggestion made by Mr McCall, mechanic at the Physiological Institute there.

NOTICE BY THE EDITOR OF THE *PROCEEDINGS.*

The blocks of all illustrations in the *Proceedings* up to 1921 are stored at the University Press, Cambridge. They will be returned to the authors if application is made to the Press during the next three months. Blocks which have not been claimed by April 1924 will be destroyed.

PROCEEDINGS

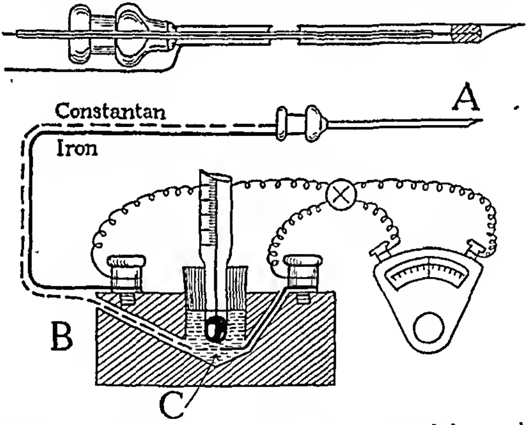
OF THE

PHYSIOLOGICAL SOCIETY,

December 15, 1923.

A needle thermo-junction. By E. D. ADRIAN and C. F. WATTS.

In many experiments it is important to know the temperature within the substance of an organ, *e.g.* a muscle or gland, and for this the most suitable indicator is a thermo-junction made in the form of a needle. The following arrangement is easily made up and is less liable to damage than the ordinary form of junction. A thin constantan wire



(No. 30 gauge), insulated except at the tip, is passed down a hollow steel needle of the type used for exploring or intravenous injection (Parke Davies, No. 24, about 1 mm. external diam.). The bared end of the wire is soldered into the end of the needle just behind the point. This forms a constantan-steel junction (*A*). A steel or iron wire is soldered to the base of the injection needle, and this and the constantan wire are led away from the needle through a thin rubber tube. The arrangement of the other junction depends on the range of temperature

which is to be covered. For working over a wide range the wires are led to a vulcanite block *B* which contains the constantan-iron junction *C* in a cavity drilled in the block and filled with oil at room temperature. The temperature of the oil is given by a small thermometer. The iron wire circuit is interrupted by two terminals from which ordinary flexible wires lead, through a reversing key, to a low resistance pointer galvanometer (moving coil type, resistance 12·4 ohms).

Over a range from 0 to 40° C. the readings of the galvanometer are very nearly directly proportional to the difference between the temperature of the two junctions. With the galvanometer at its greatest sensitivity a difference of 1° C. gives a deflection of 4 scale divisions. For ordinary purposes we have reduced this by a shunt so that 1° = 1·5 scale divisions. The instrument then covers a range of about 35° without preliminary adjustment and with an accuracy of at least ½°. For more accurate measurement between 30 and 40° a thermos flask containing water at 35° is substituted for the vulcanite block.

The advantage of this form of thermo-junction is that it is protected from damage and that it can be introduced into any part of the body which can be reached by an exploring needle. The cutting edges of the needle can be sharpened without difficulty so that its introduction should be no more painful than that of an exploring or injecting needle. Junctions of this type and the other parts of the apparatus can be obtained from the Cambridge and Paul Instrument Company.

On the effect of insulin on the isolated intestine of the rabbit.

By L. B. Winter and W. Smith.

The rabbits were killed and pieces of the small intestine were placed in warm oxygenated Ringer solution as quickly as possible.

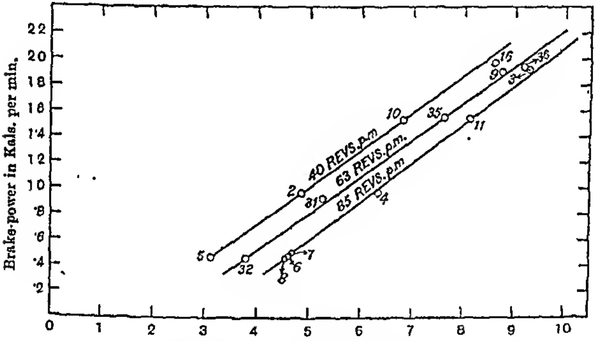
A portion was then connected to a recording lever, and tracings of the contractions obtained. On adding insulin (crude or hydrochloride) to the solution a diminution in the amplitude of the beats takes place, often accompanied by diminished tonus of the muscle. After a variable interval the normal beat and tonus is regained, or it may be quickly restored by addition of pituitrin or pilocarpine. On adding atropine to the fluid the subsequent addition of insulin has little or no effect on the contraction. The action of the insulin would appear therefore to be on the nerve cells or endings rather than directly on the muscle. Since adrenaline is effective after atropine, insulin would appear to be acting as a depressor of the vagal system in the gut and not as a stimulant to the inhibitory sympathetic endings.

Relationship between speed and efficiency.

By F. A. Duffield and J. S. Macdonald.

Benedict and Cathcart, in their comprehensive study of "Muscular Work"(1) found the "gross efficiency" greater the slower the rate of cycling. Their professional subject (M.A.M.) exhibited a maximum value at the slowest rate of movement habitual to him, 70 revolutions per minute—a slow rate, surely, only in the case of this professional subject. The increase of efficiency with diminution of rate of movement was such as to lead them to say that "a still slower rate of movement might have provided an instance of still greater efficiency."

It is clear that whatever use they may have made of the term "optimum efficiency" no practical acquaintance was made with a



value from which there was a decline on either side of a nodal experience, or to speak briefly, they did not, as a matter of fact, observe an "optimal efficiency." This is a point of genuine interest to accountants of the data of "movement" who have to make allowances for expectations based on the nature of muscular contraction, or—more important still—expectations based on time factors due to the use of long levers in muscular movement. It is also a point which, it would seem, has failed to attract due notice(2).

We have for some time studied the CO_2 output and O_2 intake of subjects cycling at varied rate against various loads (values of "brake-power") and have found the "gross efficiency" increase at much slower rates, and have also failed to reach any optimal value of "gross efficiency."

Thus with the subject "Harrison" (age 23, weight 64·4 kilos, height 5 feet 7½ inches) the "gross efficiency" is maximal at 40 revolutions per minute.

The results however are such as to claim expression in another fashion, and thus indicate statement in terms of "gross efficiency" as beside the point. They are contained in the accompanying chart, in which the ordinate represents the measured "brake-power," or "load" and the abscissa the associated "heat-production" as deduced from the O_2 intake, and "the respiratory quotient."

It is seen that the results fall naturally into three main lines, each one related to a particular rate of movement. Thus the influence of speed is self-apparent, and since, in addition, the lines are practically parallel and the "slope" thus the same in each case, the results are expressible in very simple fashion,

$$Q = \theta K + \phi V,$$

where Q is the heat-production, V the rate of movement, K the load or "brake-power," and where θ and ϕ are practically constants. That is to say the results are expressible as a total cost partly assignable to the work done *at a practically constant efficiency*, and partly to the "cost of movement" at a particular rate(3).

In these particular experiments it is seen clearly that the "cost of movement" varies in the most simple fashion with the rate of movement. This we attribute to the nature of the "cycle" used in these experiments, its "back-wheel" being heavily loaded to form a "flywheel," differing thus very definitely from the "copper disc" used by Benedict and Cathcart, and also by us in our previous experiments.

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Reaction of urine. By E. M. Watson.

Dodds(1) has given reasons why, in all studies of variations in the reaction of the urine, the influence of secretions into the alimentary canal should be considered. It is possible, till the contrary is proved, that if some subjects show no alkaline tide after meals, it is because they are among the 4 p.c. of normal persons who, as Bennett and Ryle(2) found, show complete achlorhydria. Equally, it may be the case,

till the contrary is proved, that a specially active secretion of gastric juice is the cause of the very marked morning alkaline tide found by Leathes(3) in subjects kept in bed and given one drink of water, the water in that case stimulating secretory activity

In all of the four cases in which I collected specimens of gastric fluid simultaneously with specimens of urine from fasting subjects before and after drinking one half litre of water, the morning alkaline tide had appeared before the water was taken, and in only one was any appreciable amount of hydrochloric acid found in the stomach until later, apparently secreted as a result of the drinking of the water

In one experiment, 500 c c of water was taken at 8 30 A M, when the alkalinity p c of the urine had already gone up from 44 to 60 It rose higher in the three succeeding hours The fluid in the stomach at 8 30, just before drinking the water, contained merely a trace of hydrochloric acid (2 c c $N/10$ p c)

In the same subject, on another day, the stomach which then contained no hydrochloric acid, was washed out with atropine at 8 30, an hour later, with no hydrochloric acid in the stomach, the alkalinity of the urine had gone up to 85 p c 500 c c of water was then taken and while small quantities of hydrochloric acid were subsequently found in the stomach (14, 8, and 32 c c $N/10$ p c in the three following hourly samples) there was no further change in the reaction of the urine In no case, therefore, was any relation between the fasting morning alkaline tide and gastric activity to be detected

The alkaline tide described by Bence Jones(4) in 1845 and 1849 as following dinner at 6 p m as well as breakfast at 8 a m did not occur, in the former case, till 2 or even 4 hours after the meal He appears to have taken no meal in the middle of the day Campbell(5) also describes an alkaline tide as the rule about three hours after any meal Dodds(6) showed that the alveolar CO_2 rises to a maximum $\frac{1}{2}$ to 1 hour after food and then falls to a minimum $1\frac{1}{2}$ to 2 hours after food, the rise, in all of a variety of experiments, appearing to be due to removal of acid from the blood by the stomach, the fall, to secretion of alkali below the pylorus

If effects of these secretions on the reaction of the urine occur, they should both occur at the same intervals after food In the change in the reaction of the urine brought about by hyperpnœa, which Leathes showed to occur, there is no lag

Dodds, however, in addition to Bence Jones and to Campbell, ascribes to activity of digestive glands changes in the reaction of the urine which occur at very different intervals after food from these, the

alkaline tide lasting on to the time of minimum alveolar CO_2 tension traced to alkaline secretions into the intestine and an acid tide much later than this. The changes in the reaction of the urine, therefore, which he ascribes to the same causes as the changes in alveolar CO_2, come on too late and last too long. This is how he accounts for the absence of an alkaline tide after lunch. He has recorded no observations as to the effect of the evening meal, nor has he recorded observations on the effect of breakfast on alveolar CO_2.

I propose to give in a later communication the results obtained in the evening hours and here those obtained on five days at the beginning of the day.

On all five occasions, the alveolar CO_2 tension obtained immediately on waking was considerably higher than later, as described by Leathes. On two of the three days, on which the usual breakfast was taken and the ordinary routine of work followed, small changes in the reaction of the urine synchronised with changes in alveolar CO_2 such as Dodds has found to follow the mid-day meal, slightly increased alkalinity immediately after breakfast, slightly diminished an hour later. On the third day, the first sample of air was taken perhaps too soon after breakfast, 15′, to show the rise of alveolar CO_2, but the sample an hour later showed a fall that may be associated with alkaline secretions into the intestine; but on this day neither the alkalinity p.c. nor the titratable acidity showed any corresponding change and the titratable acidity indeed rose in the first hour after breakfast and again in the second.

On none of the days did the much larger morning alkaline tide occur simultaneously with the gastric changes in alveolar CO_2 tension; on one day it preceded breakfast and on two days when no breakfast was taken it was more conspicuous still.

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Some peculiarities of mitral insufficiency, clinical and experimental. By D. T. Barry.

Optical records of cardiogram and arterial and venous pulses in healthy and abnormal conditions have been made from man. Records of mitral regurgitation yield evidence of backflow in early systole before

the semilunar valves open. This early leak is considered by Wiggers and Feil[1] to be of quite trivial amount or non-existent in experimental work. Straub[2] and Schwartz[3] hold that leakage may be free in the presphygmic period.

The present writer has investigated the question by producing mitral leak in the heart lung preparation and has obtained records which strongly support the view that regurgitation may be free in the early phase of systole, especially with large leak and low auricular pressure.

A mechanical factor in pulsus alternans is suggested, namely efficient and inefficient closure of the A.-V. valves by alternating volume in the ventricle. This suggestion is supported by a comparison of experimental records of pulsus alternans with clinical curves of the same. Another conclusion which follows from these records is that in premature beats with which the auriculo-ventricular valves are not floated into position early regurgitation is free, and the premature beat accordingly cannot aid in compensation.

Toxic action of Mercapturic Acids. By T. S. HELE and E. H. CALLOW.

In the literature there are two records of the administration of brom-mercapturic acid by mouth to dogs. Baumann and Preusse (1881) gave 8 gm. of the ammonium salt. They recorded no ill-effects. No glycuronic acid was excreted and only traces of mercapturic acid. It is possible that the dose was not absorbed. Marriott and Wolf (1907) gave 1 gm. of the acid, but the sulphur analyses of the urine do not show clearly whether the mercapturic acid was absorbed or not.

In order to complete our studies on the metabolism of the halogen derivatives of benzene, we gave mercapturic acids by the mouth to discover whether the body can oxidise these substances. Four dogs of 6 to 7 kilos in weight have been used for our experiments, which are of a preliminary character. We found that half a gram of brom-mercapturic acid was harmless, whereas 1 gm. produced after a delay of about 8 hours a very marked hæmoglobinuria. The onset was sudden and the recovery rapid. Two gm. of chlor-mercapturic acid had the same toxic action. One gm. of the ammonium salt of brom-mercapturic acid given subcutaneously also produced a hæmoglobinuria,

[1] *Heart*, 9. 149. 1922.
[2] *Deutsch. Arch. f. Klin. Med.* 122. 1917.
[3] *Wein. Klin. Wochen.* 18. 1905.

while half a gram of the ammonium salt of chlor-mercapturic acid was harmless.

The action is remarkable, when the comparatively non-toxic character of brom- and chlor-benzene is considered. These dogs are capable of dealing with 5 gm. of brom-benzene daily without any suggestion of *a hæmolysis being produced, that is ten times the dose* of *brom-benzene* equivalent to the toxic dose of brom-mercapturic acid. And yet the formation of a mercapturic acid is called a protective synthesis.

What is the explanation? Baumann and Preusse found that the mercapturic acids are excreted in combination with glycuronic acid. It is possible that the formation of mercapturic acids is the result of the toxic action of the halogen benzenes on the cell and that the products have marked hæmolytic properties. This may be prevented by the union of the toxic bodies with glycuronic acid. The velocity of the second reaction may be greater than that of the first reaction, so that hæmolysis never follows the administration of halogen benzenes.

At present we are preparing the glycuronic acid compounds with the view to determining the relative hæmolysing power of these compounds and the uncombined mercapturic acids.

We have carried out preliminary experiments with quinine to determine whether it has any effect on sulphur metabolism, as this compound is sometimes associated with the onset of Blackwater Fever in Malaria. Our experiments up to the present have been negative, but further work is in progress.

The Active Principles of Extracts of the Posterior Lobe of the Pituitary. By N. B. Dreyer and A. J. Clark.

Hogben and Winton(1) studied the action of extracts of the posterior lobe of the pituitary gland in producing darkening in frogs; they concluded that the uterine and melanophore stimulants were practically identical, and suggested that the action of pituitary extracts on frog's melanophores might serve as a basis for the physiological standardisation of pituitary extracts.

These conclusions are rendered doubtful by their observation(2) that the extracts of the Pars Intermedia of the ox pituitary have a more intense action upon frog's melanophores than extracts of the posterior lobe, since Herring(3) found the reverse to be true as regards the action of such extracts on the isolated uterus.

The writers found that the melanophore and uterine stimulants of the pituitary could be partially separated by ultrafiltration through collodion sacs Collodion membranes in thimble form were used and were made from Necollodion containing 9 p c pyroxylin in equal parts of alcohol and ether Filtration was performed under a pressure of 100 cm of water The membranes under these conditions retained from 80–95 p c of congo red (0 5 p c solution) The filtrate was found to have practically no action on the colour response of frogs but to have quite a strong action on the isolated organs

Exp 1 Thick membrane used which retained 95 p c congo red

Pituitary extract 1 in 5000 dried gland ultrafiltered

Darkening was produced in frogs by one twentieth c c original fluid, but no action was produced by 1 c c of the ultrafiltrate

When tested on the isolated uterus of the guinea pig the ultra filtrate was found to have about a quarter of the oxytocic action of the original fluid, and on the decapitated cat the ultrafiltrate was about one sixth as active as the original fluid in raising the blood pressure

Exp 2 Pituitary extract containing 1 p c of dried gland filtered through membrane which retained 80 p c congo red

The following amounts produced colour change in frogs

Original fluid 0 0005 c c (equals 0 005 mgm dried gland) produced a marked action, while ultrafiltrate 0 01 c c (equals 0 1 mgm dried gland) produced only a slight action

In the perfused isolated ear of the rabbit the injection of 1 c c of diluted original fluid corresponding to 0 002 p c dried gland produced 60 p c diminution in the flow, while the same amount of ultrafiltrate produced 86 p c diminution

Tests on the isolated uteri of the rabbit and guinea pig showed that the oxytocic action of the ultrafiltrate was about one half that of the original fluid

These results show that the melanophore stimulant is almost completely retained by ultrafiltration through membranes which only retain a portion of the oxytocic substances The two actions, therefore, appear to be produced by different and separable active principles, and estimation of the melanophore stimulant does not necessarily measure the oxytocic activity

It further seems probable that the vaso constrictor substance is not identical with the oxytocic substance

Further experiments are in progress

The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society

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The partial neutralisation of the acidity of the gastric contents in the stomach, the opening of the pyloric sphincter and the changes in the duodenum during digestion. By M. McC. Baird, J. M. H. Campbell[1] and J. R. B. Hern.

Fractional test meals have been done on 60 healthy medical students with the ordinary gruel meal and the Einhorn tube, according to the technique described by Bennett and Ryle(1). The usual titrations for acidity with di-methyl and phenolphthalein as indicators were done in each case, on specimens withdrawn each 15 minutes. In half of the subjects the total chlorides were also determined by Volhard's method on specimens withdrawn each 30 minutes. With certain precautions it has been found that sufficiently accurate results can be obtained without ashing each sample.

As suggested by Bolton and Goodhart(2) the curve of chloride secretion is the more accurate measure of the amount of HCl secreted by the stomach. In many cases the curves for chloride and acidity respectively show that considerable amounts of acid are neutralised in the stomach.

By passing one tube into the stomach and a second into the duodenum before the start of the meal and by applying continuous suction with a water pump to the latter, the contents of the duodenum can be withdrawn and examined during the course of digestion. The position of the second tube in the duodenum was confirmed by X-ray examination.

The rate of emptying of the stomach is not affected by this proceeding and nothing unpleasant is noticed after the tubes have once been swallowed. This is some evidence that the normal course of events is not entirely upset. Practically the same method has been used independently for a different purpose by Lim, Matheson and Schlapp(3).

With this method regurgitation from the duodenum into the stomach is effectively prevented in most cases, as is shown by the absence of bile in the gastric contents, when it is present in large amounts in the duodenum. Contrary to our expectation the partial neutralisation of the acidity of the gastric contents in the stomach takes place in just the same way in these cases. As precautions were taken to prevent the swallowing of saliva, this must have been due to the secretion of alkali by the stomach. The possibility of such a secretion from the pyloric part of the stomach was shown in several much earlier experiments(4).

This method of simultaneous gastric and duodenal sampling (the

[1] During the tenure of the Hilda and Ronald Poulton Fellowship at Guy's Hospital.

latter being continuous) gives a picture of the passage of food from the stomach to the duodenum and of the changes which take place there. The conditions are at least as normal as those after a fistula has been established, and this method can be readily applied to man.

After drinking water or gruel, some passes through the pylorus within the first minute, and bile nearly always appears in the duodenum during the same period. The first secretion of bile in man must therefore be under reflex rather than chemical control. Generally the acidity of the gastric contents is neutralised as soon as they reach the duodenum and their reaction becomes alkaline to litmus and di-methyl, but remains acid to phenolphthalein. Even under normal conditions the neutralisation is not always as complete as this.

In more abnormal conditions the duodenal contents may be strongly acid. One subject drank 200 c.c. $N/10$ HCl. During the first 15 minutes not very much of the acid left the stomach, and the portion which did pass the pylorus was completely neutralised in the duodenum. During the next 5 minutes 67 c.c. $N/20$ HCl was recovered from the duodenum, *i.e.* the pylorus was widely open when the duodenal contents were acid. This degree of relaxation of the sphincter cannot have been due to the presence of the tube, for little had passed previously—only 13 c.c. during the first 5 minutes. This experiment confirms the view of the older physiologists, which is still held by many clinicians and radiologists, that the opening and closing of the pylorus may be controlled by other factors than the acidity of the duodenal contents.

A full account of these experiments will be published in the next number of *Guy's Hospital Reports.*

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Observations upon capillary pulsation. By THOMAS LEWIS[1]. (*Preliminary Communication.*)

If the hand of a young and normal subject is soaked in water at 45° to 47° C. for three minutes, pulsation in the skin colour, of cardiac rhythm, can always be elicited by pressing a sheet of glass upon the finger tips, and often it can be elicited in many portions of the palm

[1] Working on behalf of the Medical Research Council.

and back of the hand. The pulsation is the same as that termed "capillary pulsation" clinically. It may be produced in almost any surface of the skin or in the mucous membrane of the lip by the method described, and frequently occurs spontaneously in the young when the skin area presenting it is warm from any cause. The application of heat to a few square millimetres of skin will suffice to induce capillary pulsation in the area so heated, one evidence of several that the phenomenon is determined by dilatation of the arterioles. Any of the known methods of widening skin arterioles (amyl nitrite, etc.) will bring about capillary pulsation. In middle life and more advanced years the reaction to heat, above described, is usually reduced or absent; suggesting that the arterioles have lost, partially or completely, their power to expand.

Clinically, "capillary pulsation" is seen most frequently and vividly in cases of aortic regurgitation, as is well known; and occurring in this condition is usually ascribed to large pulse pressure. It can be shown that large pulse pressure is not the chief determining factor even in aortic disease, though no doubt it aids. There is but an imperfect relation between the high pulse pressure in the brachial artery and capillary pulsation in the fingers. By placing an armlet on the upper arm and maintaining its pressure at a level equal to or above normal diastolic pressure, the pulse pressure in the *lower* arm of an aortic case can be reduced to normal limits; in these circumstances, and given full pulsation originally, capillary pulsation in the fingers persists. Capillary pulsation in aortic cases appears to be confined to those skin areas which are at the time warm. It is often conspicuous in the forehead and cheeks, and these are then warm or hot to the touch; closely adjacent areas of skin if cool or cold, even though the vessels in them are equally full, present little or no pulsation. If the cheeks are of equal colour, but one is found to be warm and the other cold, capillary pulsation is seen on the warm side only. If those areas of skin which are red are examined over the body generally, the same relations between skin temperature and pulsation appear to be constantly observed. The customary clinical methods of displaying capillary pulsation to advantage, namely, stroking the skin heavily with a blunt point or rubbing it, are methods which locally dilate the skin arterioles. Capillary pulsation in aortic regurgitation is determined chiefly therefore by a dilated condition of the arterioles, rather than by high pulse pressure. Those areas of skin which present capillary pulsation in aortic regurgitation are areas in which there is arteriolar dilatation. This arteriolar dilatation is often, though not necessarily, accompanied by visible or palpable dilatation of the main

arteries The vaso dilatation so identified is usually confined to the head and, in lesser degree, to the upper extremities in aortic cases, it helps, in the presence of a low mean blood pressure, to maintain a sufficient flow of blood through the capillaries of these portions of the body

Amongst the vessels concerned in "capillary pulsation' are the actual capillaries, as Sumbal(2) has shown The pulsation is frequently trans mitted through these into their collecting venules, and from these further into the larger venules of the sub papillary plexus The pulsation in the veins, when traced to its source, has always been centripetal These events can be made out microscopically most clearly in areas of skin which show well defined clinical "capillary pulsation' and from which the horny layer of the skin has been removed previously by applying a blister Pulsation of the minute venules of the face is usually to be identified in cases of free aortic regurgitation both macroscopically and microscopically Thus, the pulsating skin colour is due in part to pulsating capillaries and in part to pulsating venules as Boas(1) has lately suggested

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(1) Boas Archives of Internal Medicine, 29 763 1922
(2) Sumbal Heart, 10 271 1923

PROCEEDINGS

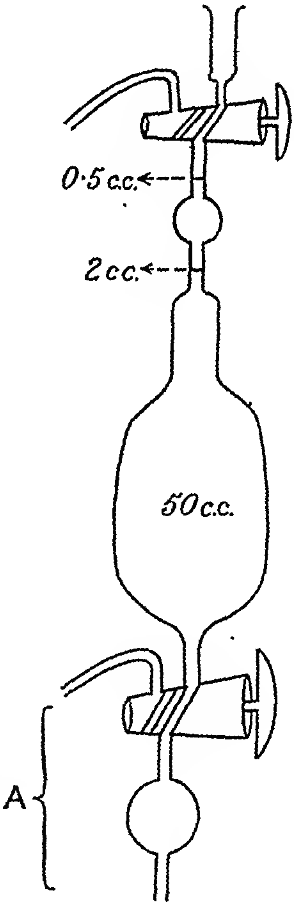
OF THE

PHYSIOLOGICAL SOCIETY,

February 16, 1924.

The accurate gasometric determination of small quantities of oxygen. By C. R. HARINGTON.

An apparatus has been devised on the basis of the Van Slyke constant volume gas analysis apparatus(1), by which it is possible to determine the oxygen content of blood with more accuracy than has hitherto been obtainable.



The only difference from the machine of Van Slyke consists in the provision of a special trap and two-way stop-cock at the lower end of the gas pipette (see diagram A). This arrangement makes it possible, after having liberated the gases from the blood in the usual way, to remove the reagent mixture from the machine. The determination of oxygen or carbon dioxide or both can then be proceeded with in the usual way with the advantage that the machine is in a clean condition. The two pressure readings are taken over a measured amount of a mixture of equal parts of glycerol and saturated sodium chloride; this obviates the necessity for the correction for reabsorption of gases which was a disadvantage of Van Slyke's first method.

By means of this machine it has been possible to standardise the oxygen analyses absolutely by making successive gasometric and titrimetric determinations of air-free solutions of hydrogen peroxide. In a series of such experiments the results obtained by the use of this machine and by titration with permanganate agreed consistently to within 0·1 millimol of oxygen.

Using 2 c.c. samples of blood it is easy to obtain duplicate results to within 0·1 millimol; 0·2 c.c. samples can be analysed, with no modification in technique, to within 0·2 millimol.

The machine can also be employed for determinations of carbon monoxide.

The above described machine was devised by the author in conjunction with Dr Van Slyke, while working in the latter's laboratory at the Hospital of the Rockefeller Institute, New York.

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(1) Van Slyke, D. D. Proc. Nat. Acad. Sci. 7. 229. 1921.

The effect of barometric pressure on the O_2 and CO_2 tension in air between the skin and the muscles. By ARGYLL CAMPBELL and LEONARD HILL.

Haldane and Priestley (1905)(1) demonstrated the effects of variations—646·5 to 1260 mm. Hg.—of barometric pressure upon the O_2- and CO_2-tensions in alveolar air in man. They found that within these limits of barometric pressure the partial pressure of CO_2 in alveolar air is little altered whilst the partial pressure of O_2 is increased by a rise of barometric pressure and decreased by a fall of barometric pressure. Leonard Hill (1912)(2) and Greenwood showed that the alveolar CO_2-partial pressure is unaltered even by excessive rise of barometric pressure.

Boycott and Haldane (1908)(3) published researches of the effects of low barometric pressures on alveolar air tensions demonstrating how the CO_2-tension is lowered by marked decrease of barometric pressure, *i.e.* below 550 mm. Hg. This decrease of CO_2-tension was considered by some to be due to an "acidosis" caused by a non-volatile acid not yet determined. Barcroft (1923)(4) and his co-workers recently examined this question and concluded that the fall of CO_2 in the alveolar air, is the effect of increased ventilation due to the action of O_2 "want" on the respiratory centre itself.

In the present research we have employed a new method introduced by one of us (Campbell, 1923)(5). Air was injected under the skin of a rabbit and left there until the CO_2- and O_2-tensions had come into equilibrium with the tensions of these gases in the tissue spaces. We were able to test the effects of marked changes of barometric pressure on these gases. By the kindness of Mr R. H. Davis, Managing Director, Messrs Siebe, Gorman and Co. Ltd, we were granted the use of the pressure chambers at Westminster Bridge Road, London.

Effect of high barometric pressure. Table *A* shows the results of a typical experiment in which the animal was exposed to barometric pressure increased from 736 to 1476 mm. It will be noted that the CO_2 p.c. actually found was decreased by almost a half so that the partial pressure (p.c.) after calculating for barometric pressure was not much changed. The O_2 p.c. actually found was increased so that the partial pressure was more than doubled. The fact, that the O_2 p.c. actually found was increased, is evidence of the therapeutic value of breathing O_2 at increased partial pressure.

Effect of low barometric pressure. Table *B* gives details of a typical experiment in which the animal was exposed to barometric pressure decreased from 752 to 514 mm. The CO_2 actually found was increased somewhat but the partial pressure was much decreased. The O_2 p.c. actually found was slightly decreased so that the partial pressure was much decreased.

Summary. The above results obtained with the new method of subcutaneous injection of air were similar to those obtained by established methods.

TABLE.

Time (mins.)	Barometric pressure mm. Hg.	% actually found in dry air under skin CO_2	O_2	Partial pressure % in moist air after calculation for barometric pressure CO_2	O_2
A					
0	736	6·45	2·55	6·02	2·38
20	1101	5·10	2·93	7·29	4·19
33	1476	3·97	3·00	7·70	5·82
47	1476	3·33	2·99	6·46	5·80
57	1476	3·13	3·02.	6·07	5·85
67	736	—	—	—	—
226	736	6·31	2·88	5·89	2·69
B					
0	752	7·08	2·31	6·62	2·16
7	514	—	—	—	—
81	514	8·28	2·11	5·12	1·30
96	514	8·32	1·97	5·15	1·22
104	752	—	—	—	—
333	752	7·41	2·54	6·93	2·37

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(1) Haldane and Priestley. Journ. Physiol. 32. 232. 1905.
(2) Leonard Hill. Caisson Sickness, p. 47. 1912.
(3) Boycott and Haldane. Journ. Physiol. 37. 355. 1908.
(4) Barcroft and others. Phil. Trans. Roy. Soc. B. 211. 452. 1923.
(5) Campbell. Journ. Physiol. 58. vii. 1923; and Proc. Roy. Soc. B. 96. 1924.

The influence of formaldehyde on the coagulation of blood.
By B. J. COLLINGWOOD.

In attempts to sterilise solutions of thrombin the inhibitory influence of formaldehyde on coagulation of blood became apparent.

The formaldehyde used in the following experiments was prepared from commercial formalin diluted with various amounts of normal saline and brought after dilutiou just to the alkaline side of phenol red by the addition of $N/10$ NaOH.

The following is a summary of the experiments.

I.

Freshly shed human blood + equal bulk of formaldehyde solution of various concentration.

0·04 % formaldehyde	Clot in 6 minutes
0·1 "	" 15 "
0·2 "	" 30 "
0·4 "	No clot in 60 minutes

Temp. 21° C.

II.

Freshly shed human blood + equal bulk of 0·4 p.c. formaldehyde to which thrombin was immediately added.

Instantaneous clot. Temp. 21° C.

Freshly shed human blood + equal bulk of 0·4 p.c. formaldehyde to which thrombin was added after 1 hour.

No clot in $1\frac{3}{4}$ hours. Temp. 15° C.

III.

Dry thrombin dissolved in 0·4 p.c. formaldehyde and added to oxalated human blood at various intervals of time after the solution was made.

Immediately after dissolving the thrombin	Good clot, 30 secs.
$\frac{1}{4}$ hour " "	Faint thread, 30 secs.
$\frac{1}{2}$ " " "	Thread, 10 mins.
$1\frac{1}{2}$ hours " "	Very faint thread in 10 mins., no more clotting in $1\frac{1}{2}$ hours

Temp. 16·5° C.

Conclusions. Both fibrinogen and thrombin are destroyed by formaldehyde, but in neither instance is the destruction immediate. It is an interesting question whether this destruction is due to the methylenation of amino groups, and whether such groups in fibrinogen and thrombin are necessary for the formation of fibrin by the interaction of these two bodies.

Biological action of light, experiments on penetration and absorption. By ARGYLL CAMPBELL, A. EIDINOW and LEONARD HILL.

Dreyer and Jansen[1], using the frog's tongue, showed that rays from a carbon arc lamp (30 amp.; 45–50 volt.), with heat rays filtered off through a quartz water container and focussed, dilated in a few minutes all the vessels in the area exposed whilst stasis developed rapidly in the capillaries. The tongue was irrigated with cold saline solution to prevent the heating effect of the visible rays. We have repeated these experiments with similar results, using a small carbon arc lamp (5–10 amp.; 50–60 volt.) and taking similar precautions to exclude the temperature effect and to focus the rays by means of a quartz lens. When the ultra violet rays were excluded by a glass screen, no stasis developed in the capillaries. Exposure of the frog's tongue to a 25 amp. arc, 230 volt. produced stasis in the superficial capillaries when no focussing lens was used; in this case a carbon pole with aluminium core was employed. The tongue was irrigated to keep it cool and placed 2 feet away from the naked arc flame.

While exposure of the frog's lung and mesentery to the unfocussed total radiation from the mercury vapour lamp at 15 cm. distance produced stasis in the capillaries—the temperature effect being excluded—we were not able to produce capillary stasis in the dorsal surface of the frog's tongue or web of the foot with the unfocussed rays in this way even after several hours' exposure. On the other hand, with the rays of the mercury vapour lamp focussed by a quartz lens we were able to produce a stasis in the tongue which a glass screen prevented. It was due then to ultra violet rays.

The unfocussed mercury vapour lamp (we used Kelvin, Bottomley and Baird's 2·5 amp.) at 15 cm. distance, acting for 10 minutes on the warm white skin of the upper inner surface of the arm produces a well-marked erythema, which comes on some two hours after exposure; it also kills infusoria, exposed in a quartz tube and kept cool, in about 10 minutes. Transparency to, or penetration by, ultra violet rays comes into play as well as the biological activation of these rays by visible rays. Infusoria are killed by the ultra violet rays in which the M.V.L. is rich because they are so small that the short rays penetrate their surface and are absorbed by the nucleus and cell protoplasm.

We conclude that in the case of the human skin the longer ultra violet rays of the M.V.L. act by penetrating to the deep cells of the epidermis. Producing changes there, erythema is excited by damaged tissue cell products acting on nerve endings and capillaries.

[1] *Mitteilungen aus Finsens Lichtinstitut*, 9. 180. 1905.

The relatinn between the spectrum of, and the affinity for certain gases for, vertebrate hæmoglobin. By M. L. Anson, J. Barcroft, H. Barcroft, A. E. Mirsky, S. Oinuma, C. F. Stockman.

The following relation has been found to hold for the hæmoglobins of Man, Horse, Cat, Sheep, Mouse, Rabbit, Hen, Pigeon, Roach, Lizard, Frog and Tortoise:

If A be the position of maximum intensity of the α band in Angstrom Units and B that of the CO band, and if K be the relative concentrations of O_2/CO dissolved in a solution which contains 50 % O_2Hb and 50 % CO Hb

$$\log K = \cdot 050\ (A - B).$$

K is the measure of the equilibrium constant of the reaction and $\log K$ that of the free energy change. Therefore the displacement of this spectral band is proportional to the free energy of the reaction.

The spectra of the hæmochromogens derived from all the above bloods are identical.

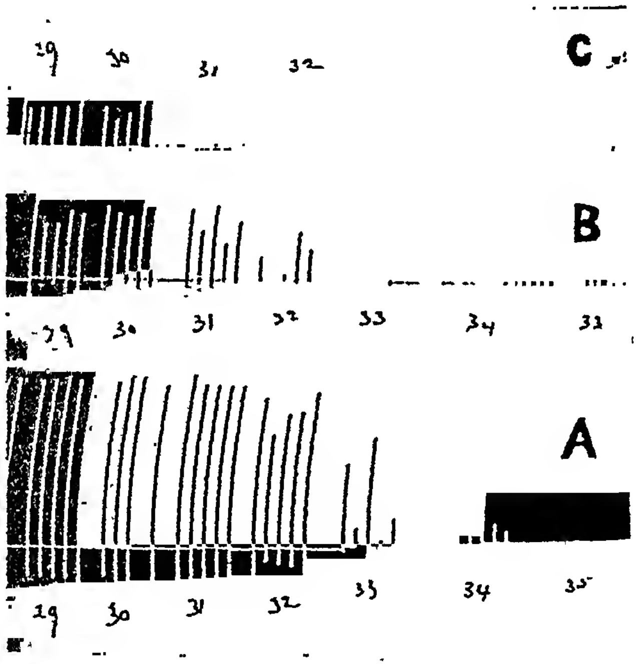
On the glycogen in the liver and muscles after insulin convulsinns. By L. B. Winter and W. Smith.

Dudley and Marrian state that glycogen almost entirely disappears from the liver and muscles of animals in insulin convulsions. Since animals may be recovered from convulsions by means of adrenaline, when the blood sugar gradually rises towards normal, it is probable that the sugar which has disappeared has not entirely been burnt. It seemed possible that the tissues of animals recovered from insulin convulsions by adrenaline might contain more glycogen than at the convulsion level.

Indications were obtained that this is so using rats. With rabbits however the glycogen may not have disappeared when convulsions occur, as much as 3 p.c. has been found in the liver of a rabbit after 24 hours starvation during convulsions. Using rabbits from the same litters greater uniformity has been obtained; while the animals of some litters may convulse at a high glycogen level, others do so when the glycogen has almost disappeared. On recovery by adrenaline it is found that the glycogen present in the liver and muscles is as a rule greater than the convulsion level (for the same litter), the blood sugar meanwhile having risen. These facts would support Noble in that insulin convulsions are not necessarily due to the absence of glycogen in the body, and further it is possible that the glycogen which has disappeared has been converted into some intermediate form from which it can be regenerated by antagonising the insulin.

On the variation in excitability produced by extension in muscle. By Grace Briscoe.

This investigation resulted from certain observations made on the diaphragm. This muscle may be regarded as consisting of two portions, the central parts forming the crura, and the lateral parts forming the dome. By moving the trunk, the tension of the muscular fibres in the two parts can be diminished or increased. If the crural fibres are put on the stretch a threshold stimulus, per the phrenic, will cause them to contract, but is ineffective with the less tense dome fibres; and *vice versa*.



(A) Foot at right angles to leg, and muscle under tension.
(B) Foot in half-way position.
(C) Foot straight with leg, and muscle slack.

The gastrocnemii, *in situ*, of rabbits and cats were investigated. Ether and urethane were given in most cases, the experiment was repeated under ether only, and in decapitated animals. An induction coil was employed, and the gradation of stimuli obtained by moving the secondary. The break shocks were sent in at regular intervals, never less than 5 seconds. The gastrocnemius and sciatic of the same side were exposed, and the muscular branches of the gastrocnemius stimulated, the connection with the C.N.S. being maintained. The muscle was not disturbed, except that a thread was tied around the tendon to record con-

tractions. The thigh was fixed at right angles to the body, and the leg shank kept horizontal. When the foot was held at right angles to the leg, the muscle was stretched, when the foot was straightened out, the muscle became slack. Thus the muscle was never extended beyond a point possible in the natural relationships of the body.

The threshold stimulus for the muscle in one position was tested, and then the foot was immediately moved to another position, and the threshold stimulus tested again, without any other alteration in the conditions. The results showed that when the foot was placed at right angles to the leg and the muscle thus put on the stretch, the excitability of the muscle was raised, and it responded to a stimulus which was ineffective when the foot was extended and the muscle slackened.

In this series ten rabbits and four cats were tested. In one cat no change in excitability could be demonstrated.

It was found that this change in excitability could not be demonstrated when the sciatic nerve was cut off from the central nervous system.

A number of experiments were also done on isolated frogs' gastrocnemius-sciatic preparations. The length of the muscle was altered by adding small axial loads. Here again a constant change in excitability could not be demonstrated.

These two observations suggest that the change in excitability produced by altering the length and tension of a muscle in its natural surroundings is dependent upon the integrity of the reflex arc, and is probably therefore a reflex phenomenon. These observations suggest that the grading of muscular response may be controlled by means of submaximal stimuli, *e.g.* in such a muscle as the diaphragm, where one part may be rendered tense and another part slackened by altering the position of the trunk (as I demonstrated in a previous communication to the Society[1]) a submaximal impulse may cause a contraction in that part of the muscle which is sufficiently under tension to be excited by it and the slack portion will remain inexcited. In this way the diaphragm may be able to meet the need for varying depths of inspiration.

[1] *Journ. of Physiol.* 54. 46. 1920.

Potassium and sodium in sweat.

By G. L. PESKETT and P. C. RAIMENT. (*Preliminary Note.*)

The following obserservations on the composition of the sweat under various conditions are interesting in connection with the results published by Moss(1).

The method of collection employed was precisely similar to that used by Moss, except that the clothes were extracted with the washings by means of a Soxhlet apparatus working *in vacuo*, which allowed of much saving of time in evaporation, etc. The final extracts were then made up to known volume and the potassium and sodium estimated by the method of Kramer and Tisdall (2).

The results were as follows:

Subject	Conditions of Expt.	Total Na	Total K
1	Work on bicycle ergometer for ½ hour	0·142 gms.	0·039 gms.
2	Rest in deep bath for 20 mins. at 101° F.	0·118 gms.	0·036 gms.
3	Rest in radiant heat bath for 15 mins., 110° F.	0·059 gms.	0·008 gms.
4	Rest in whirlpool bath for ½ hour at 100° F.	0·023 gms.	0·037 gms.

It will be seen that with the exception of the figures obtained in the last experiment, which is still under investigation, the results do not agree with the observations of Moss, as regards the relative proportion of Na and K.

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(1) Moss, K. N. Proc. Roy. Soc. B. 95. 181. 1923.
(2) Kramer and Tisdall. J. Biol. Chem. 91. 263. 1920.

NOTICE BY THE EDITOR OF THE *PROCEEDINGS*.

The blocks of all illustrations in the *Proceedings* up to 1921 are stored at the University Press, Cambridge. They will be returned to the authors if application is made to the Press. Blocks which have not been claimed by April 1924 will be destroyed.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

March 15, 1924.

The nature of the muscular rigidity and tremor of paralysis agitans. (*Preliminary observation.*) By F. M. R. WALSHE.

Loss of voluntary power and hypertonus, or spasticity, follow lesions involving the pyramidal system of fibres in man. The physiological identity of spasticity with experimentally produced decerebrate rigidity is now generally recognized. In paralysis agitans and other extra-pyramidal motor diseases a form of muscular hypertonus is present which differs in certain important respects from the spasticity of pyramidal system lesions. Very little is known of the physiological significance and origin of this extra-pyramidal muscular rigidity. It is nearly always accompanied by tremor, but never by any definite paralysis of voluntary power. De-afferenting the spastic muscles in pyramidal system disease, by the operation of posterior root section, renders them atonic, an indication that spasticity is a proprioceptive reflex arising in the muscle itself. For obvious reasons, this operation has not been employed as a therapeutic measure in paralysis agitans, and we do not know whether the rigidity of this disease is such a proprioceptive reflex, or indeed is reflex at all. Its relationship to tremor is equally unknown, and it has not been possible to determine to what extent it is a factor in producing the other disorders of movement characteristic of paralysis agitans.

Liljestrand and Magnus have found that the tonic muscles of decerebrate rigidity or of tetanus spasm can be de-afferented and rendered flaccid by doses of novocain which when injected directly into the muscle do not impair conduction along the efferent motor nerves.

This method has been applied to the study of rigidity and tremor in paralysis agitans. The integrity of the motor nerves is estimated by periodical examinations of voluntary power, using a spring balance as described by Lovett in his 'spring balance muscle tests.' A one per cent. sterile solution of novocain is injected into the motor point of the muscle.

The adequate dose varies with the bulk of the muscle under investigation. From 18 to 25 c.cms. for biceps, 10 c.cms. for each head of triceps, and the same dose for each of the forearm muscles are found to give most satisfactory results. Within ten minutes of the injection, the rigidity, as tested by passive movements, begins to wane, and may disappear completely within twenty minutes, leaving the muscle flaccid. Voluntary power remains intact. In these circumstances, the muscle becomes painless to pressure, its tendon jerk may be completely abolished, and certain changes in voluntary movement are observed. The range and speed of such an alternating movement as flexion-extension of the forearm are both increased, sometimes to double the original amplitude and rate, fatigue is delayed, and the subject may be able to carry out purposive movements which have long been impossible. In some cases slight ataxy of movement was noted. The following conclusions may be drawn: the rigidity of paralysis agitans is a proprioceptive reflex arising in the rigid muscle, it is a potent factor in producing the slowness and limitation of range of movement which are so characteristic of paralysis agitans. Tremor is a distinct and separable phenomenon, and persists unabated in the flaccid de-afferented muscle.

A method of obtaining 50 c.c., or more, of human arterial blood.

By F. R. FRASER, G. GRAHAM, and R. HILTON.

The method of obtaining arterial blood by puncturing the radial artery, as introduced by Hurter(1) and Stadie(2), has been of great value in the investigation of the respiratory problems in man. By this method it is not easy to obtain sufficiently large amounts of blood to allow of the necessary observations for the construction of CO_2 dissociation curves, etc. In some patients it is possible to obtain the necessary quantity from the brachial artery at the elbow. By puncture of the femoral artery, however, we have found that it is easy to obtain 50 c.c., or more, of arterial blood without danger to the patient and usually without any pain or inconvenience.

The femoral artery immediately below Poupart's ligament is palpated, and the skin over the place where the pulsation is most distinct is anaesthetised with novocain. We have used an all glass 50 c.c. syringe containing 0·25 grm. of dry powdered potassium oxalate and sufficient liquid paraffin to fill all the dead space, and a platinum-iridium needle 2·5 cm. long and 20 w.g. size. The needle, sterilised and fitted to the syringe, is pushed through the skin and gradually advanced inwards and

slightly upwards until the artery is felt pulsating at the point of the needle. It is then pushed gently into the artery and the blood immediately fills the syringe, pushing out the piston. When the syringe is filled, the needle is withdrawn and the patient told to maintain firm pressure over the artery for a few minutes. The patient should continue to lie in bed for the rest of the day.

We have now carried out this procedure more than forty times. Occasionally there is a very slight pain as the needle is approaching the artery, but there is no pain after the operation is completed. One of us got up and continued his day's work immediately after a puncture had been performed on him, and there was some effusion of blood, as evidenced in a greenish blue discolouration, and a slight ache for some days after, but this has not been observed in the patients who are confined to bed.

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(1) Hurter. Deutsch. Arch. klin. Med. 108. 1. Leipzig, 1912.
(2) Stadie, W. C. Journ. Exper. Med. 30. 215. New York, 1919.

Solubility of oxyhæmoglobin in the red corpuscle.

By Gilbert S. Adair.

The following quotations are from Starling's *Principles of Human Physiology* (1915). "Though the hæmoglobin can be separated from the stroma by very simple means, it is difficult to believe that it is in watery solution...the solution would have to contain at least 30 p.c. hæmoglobin. No solution of this strength can be prepared."

In some of my experiments on osmotic pressure at the Massachusetts General Hospital, part of the hæmoglobin crystallized out, thus giving an opportunity for solubility determinations.

Exp. 74. (pH 7·0, salts 0, temp. 4°), pressure 22 mm., solubility 6 p.c.

Exp. 174. (pH 8·0, salts ·23, temp. 4°), pressure 252, solubility 27·3 p.c.

It is quite true that the solubility of pure hæmoglobin in pure water is much less than 30 p.c., but in a mixture of salts resembling the salts inside the corpuscle, hæmoglobin crystals dissolved readily.

Starling suggests that some form of combination is necessary in the corpuscles to prevent the hæmoglobin from crystallizing out. This combination is potassium hæmoglobinate, and Exp. 174 shows that the solubility of this compound would be large enough to account for the absence of crystals in the corpuscles, even at fairly low temperatures.

'After-discharge' in a peripheral nerve-muscle preparation as influenced by the state of the circulation and the initial passive stretch. (*Preliminary note.*) By JOHN FARQUHAR FULTON.

If the unexcised semitendinosus, sartorious, or gastrocnemius of the frog is stimulated under various degrees of initial stretch through its cut nerve by 50 break shocks delivered at 70 per sec., so long as the circulation remains active the following features may be observed (recorded by an "isometric" lever permitting of 2–3 mm. tendon movement): (1) the greater the initial tension within physiological limits, (*a*) the greater is the height of the tetanic plateau, an observation similar to that lately made by Sherrington and Liddell(1) upon the responses of mammalian muscle, (*b*) the longer the time of ascent to the 'steady state' or plateau, (*c*) the greater the extent of the peripheral after-discharge. (2) 'Half-relaxation' of a muscle with active circulation is usually achieved within ·01 sec. after the termination of the after-discharge (see Fig. 1). When the circulation is stopped relaxation becomes slow and irregular. (3) The temperature coefficients for the ascent time and for the after-discharge vary together from 1·4 to 1·5 (see Fig. 2). (4) The ratio between the duration of the after-discharge and the time of ascent in gastrocnemius appears to be a constant approximating 0·50 for all degrees of initial stretch up to 130 gms., and for all temperatures between 10° and 25°. (5) The gastrocnemius at 20° is able to follow mechanically 70 per sec.

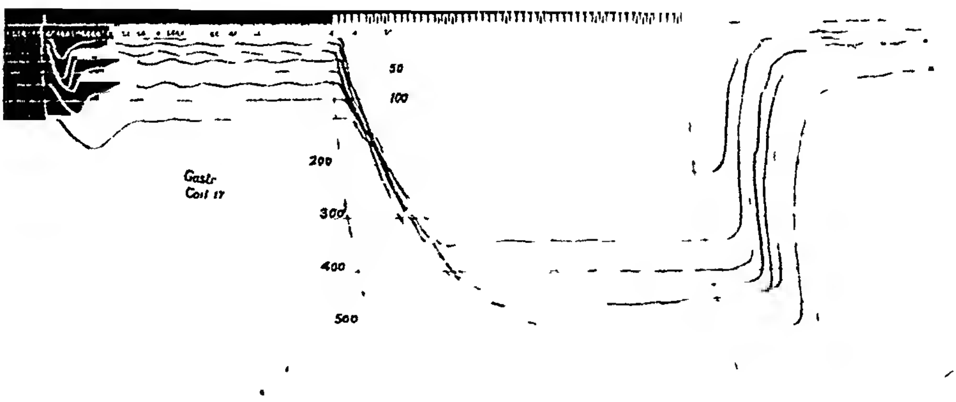

Fig. 1. Unexcised *gastrocnemius* of 20 gm. frog at 20° C. Stimulated by 52 just maximal break shocks delivered through cut sciatic nerve at 70 per cent. by a Sherrington torsion-wire separation key. A single shock precedes the tetanus. Coil coreless and secondary in series with 20,000 ω graphite resistance. Preparation spinal; artificial respiration; circulation active. Stimuli shown by upper signal; time in ·01 sec. by the lower. Tendon movement ×18.

From these observations it is concluded (1) that the delayed relaxation of the twitch with increasing initial tension is an expression of greater initial lactic acid production (cf. Fenn(2)), rather than to its less rapid elimination(3). (2) The plateau represents a state in which the *rate* of lactic acid production is exactly equalled by its rate of utilization. Therefore, when the rate of production (the *amount*, i.e. for each separate stimulus) increases, more time is required to reach the steady state or plateau, and since this greater velocity of reaction implies a more prolonged acid production after cessation of the stimuli, we have here the *raison d'être* of the after-discharge.

Fig. 2. Unexcised *gastrocnemius*. Conditions similar to those indicated in Fig. 1 save that the temperature is varied, and the preparation is decerebrate respiring normally. Initial tension 50 gms. for each curve. Tendon movement × 18.

TABLE I. Showing the effect of increasing the initial tension upon the ascent time and the after-discharge. From a typical record shown in Fig. 1.

Initial tension (gms.)	Tension developed	Ascent time (·01 sec.)	After-discharge (to ½ relax.)	Ratio after-d./ascent t.
5	215	14	8	0·57
15	350	20	10	0·50
25	410	21	11	0·52
40	430	24	13	0·54
55	470	30	15	0·50
70	480	32	16	0·50
100	520	40	20	0·50
130	560	49?	23	0·48?

TABLE II. Showing the effect of temperature on the ascent time and the after-discharge. Initial tension 50 gms. Taken from Fig. 2.

Temperature tension	Tension developed	Ascent time		After-discharge		Ratio after/ascent
10	550	43	coef. 1·48	19	coef. 1·40	0·50
15	535	35		16		0·46
20	535	29		13·5		0·46
25	520	22?		11		0·44?

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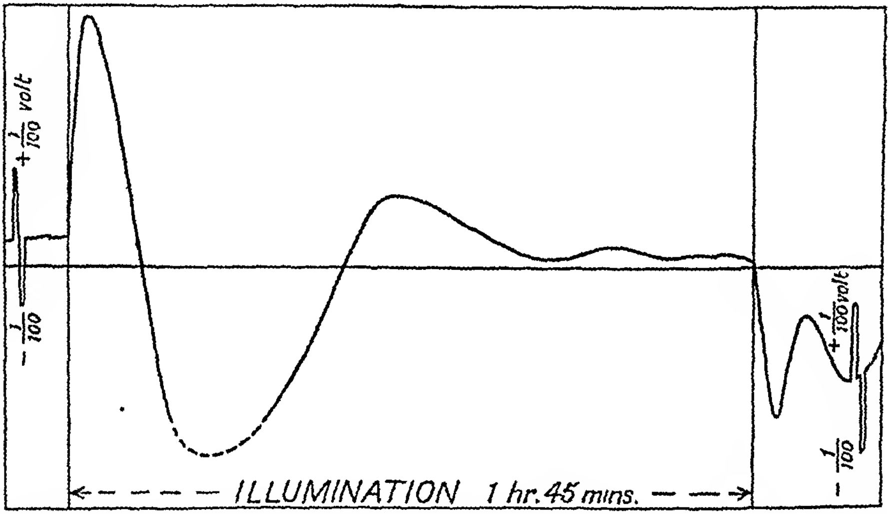
On the fluctuations of potential in green leaves under the influence of light. (*Preliminary note.*) By J. C. WALLER.

When a green leaf is illuminated, changes of electrical potential are brought about. These changes can be made evident by comparing the potential of an illuminated area with that of a control area kept at constant potential.

As the control we may use *either* a shaded portion of a normal green leaf *or* an albino portion of a variegated leaf; this is part of the evidence that it is the activity of chlorophyll which plays a predominant part in producing the photo-electric response of leaves.



Fluctuations of potential in young leaf (attached to plant) of cabbage (*Brassica oleracea* L.) during and after $1\frac{3}{4}$ hours' illumination by electric light, the heat of which was cut off by a water bath. Upward displacement of record indicates positivity of the illuminated area: downward displacement negativity. The horizontal line is zero. Room temperature 18° C. 23 January 1924.

The changes of potential take the form of fluctuations, positive and negative, which gradually subside if the illumination is prolonged (see Figure). When the light is cut off, there is an after-effect which also takes the form of fluctuations, resembling those which occur during illumination, but generally starting in an opposite direction, as shown in the Figure.

The fluctuations may be explained by supposing that light brings about two sets of chemical actions in the leaf, the ionic movements of which are the cause of positive and negative states respectively. According as one or other action predominates, so the electrical potential would be shifted in the positive or negative direction.

The above theory was put forward by the late Professor A. D. Waller and is an application of the well-known theory of Hering, according to which a negative effect implies "dissociation" and a positive effect "association."

The green leaf, the synthetic organ *par excellence* of Nature, appears to be well adapted for testing the theory, and affords evidence as follows:

1. The positive change (indicating synthesis) is favoured in a leaf tested in air fertilised by an addition of CO_2.

2. The positive change is also favoured in a leaf which has been kept in darkness for some hours previously to testing, and whose tissue would therefore be charged with CO_2 or other products of respiration.

3. Conversely the negative change is more pronounced in leaves which have been exposed to light for some hours previously to testing.

4. Tests in CO_2-free air are not yet decisive.

5. During illumination lasting one hour or more, the positive fluctuations are as a rule, but not always, predominant over the negative. This is in accordance with the well-known fact that in leaves exposed to light for such periods, synthesis predominates over respiration.

6. The subsidence of the fluctuations during exposure may be due to respiratory and synthetic processes coming nearer towards equilibrium with each other, but is not satisfactorily explained.

Comparison of osmotic pressures of oxyhæmoglobin, reduced hæmoglobin and methæmoglobin. By GILBERT S. ADAIR.

Douglas, Haldane and Haldane in 1912 (this *Journal*) suggested that reduced hæmoglobin (Hb) was more aggregated than oxy. (HbO_2). The following figures give p, the observed osmotic pressure in mm. mercury at 0° C. and c in grams Hb per 100 c.c. solution with an outer liquid KCl M/10, Na_2HPO_4 M/15, pH 8.

The hæmoglobin was prepared from the blood of G. S. Adair. The ratios p/c, given below, prove that the osmotic pressure is practically independent of the degree of oxidation, therefore Haldane's theory is not confirmed.

HbO_2, 6·78 p.c., p 20·2, ratio 2·98.
Hb Met, 6·78 p.c., p 19·8, ratio 2·92.
Hb 4·2 p.c., p 13·2, ratio 3·14.

The comparison of these figures is much easier than the explanation of their absolute significance, but a later article will show they indicate a molecular weight near to 66,800.

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